STUDIES TOWARDS THE SYNTHESIS OF MYCINOLIDE III, MYCINOIC ACIDS I & II AND 1β,2α-DIMETHYL GIBBERELLINS

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A thesis submitted to the University of Bristol in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Science .

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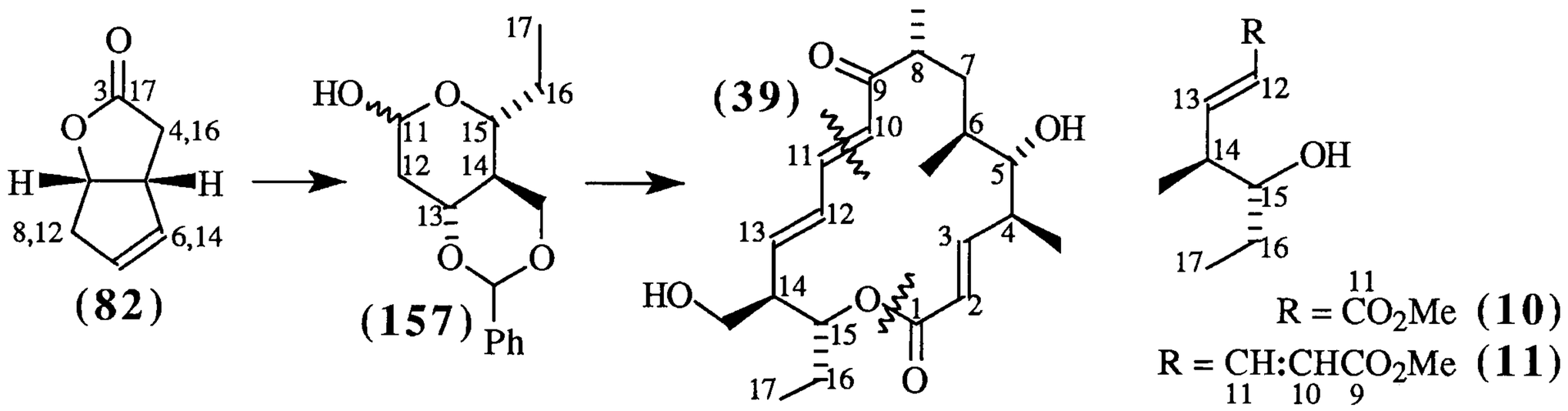
ABSTRACT

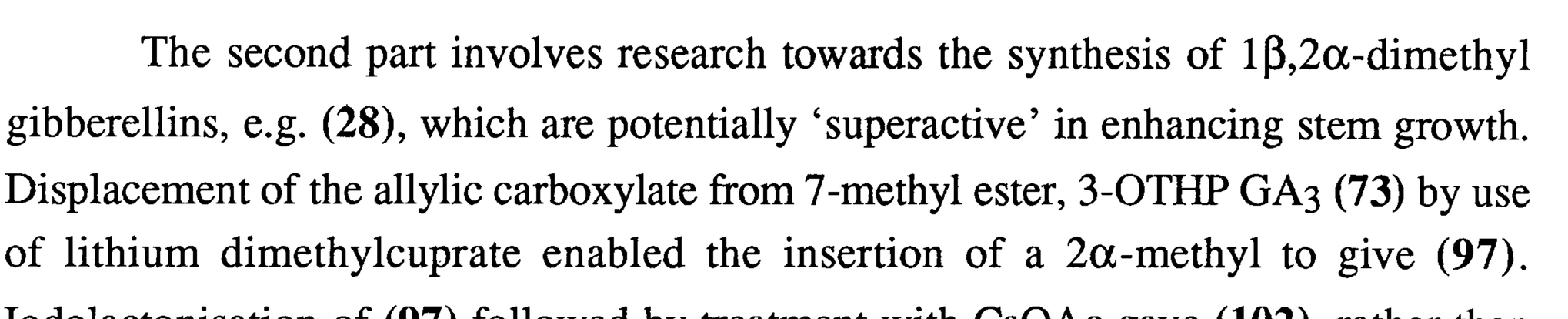
The thesis is divided into two parts.

The first project involves studies towards the total synthesis of the 16-membered ring of the macrolide antibiotic, mycinamicin III and also of the putative biosynthetic precursors, the mycinoic acids.

The rigid conformation of the starting material, bicyclo[3.3.0]octenone (**82**), ensured facial selectivity creating prochiral centres on the template, and thus directed stereoselective syntheses towards both fragments of the aglycone of mycinamicin III (**39**), and to the methyl mycinoates I (**10**) and II (**11**). The successful addition of a 4α-methyl group to (**82**) was achieved; with further manipulation this would lead to the C_1-C_{10} fragment with the methyl in the β-configuration at C-4 of (**39**). The removal of the oxygen at C-3/17 of (**82**) was required to yield the C-17 methyl functionality, thus techniques for deoxygenation were investigated. The reduced derivatives containing an ethyl group, e.g. (**157**), were treated with a variety of reagents in attempts to give the C_{11}-C_{17} fragment and hence the mycinoic acids.



The second part involves research towards the synthesis of 1β,2α-dimethyl gibberellins, e.g. (**28**), which are potentially 'superactive' in enhancing stem growth. Displacement of the allylic carboxylate from 7-methyl ester, 3-OTHP GA_3 (**73**) by use of lithium dimethylcuprate enabled the insertion of a 2α-methyl to give (**97**). Iodolactonisation of (**97**) followed by treatment with CsOAc gave (**102**), rather than the desired 1α-acetate; aqueous hydroxide did not displace the iodide, but caused epimerisation of the 3-alcohol. The ketone (**110**) was prepared from the 3,13-diacetate derivative of (**97**), *via* a peracid-mediated cyclisation and subsequent mild oxidation, as an intermediate towards the target 1β,2α-axially disubstituted gibberellin (**28**).



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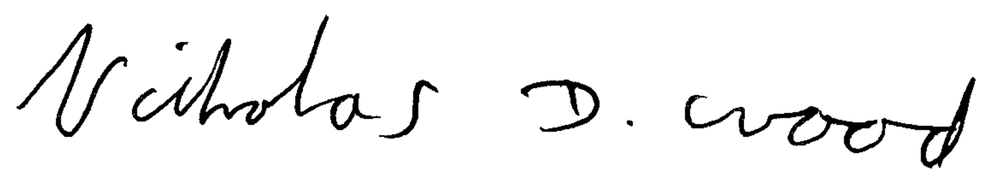
Thanks to the students of the CLW group, particularly those in laboratory E407, and also various members of TJS and JBS groups.

My apologies to those people who, through no fault of their own, had to put up with my less than sunny disposition.

MEMORANDUM

The work described in this thesis was carried out between August 1992 and November 1995 in the School of Chemistry at the University of Bristol. Except where indicated by reference, the work is original and has not been submitted for any other degree.

The views expressed in this thesis are those of the author and not of the University.



Nicholas D. Wood

June 1996

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ABBREVIATIONS

Ac	acetyl
AIBN	α,α'-azo*bis*isobutyronitrile
ap	apparent
aq	aqueous
Bn	benzyl
BOM	benzyloxymethyl
bp	boiling point
br	broad
CI	chemical ionisation $[CH_5]^+$
CSA	camphorsulfonic acid
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
de	diastereomeric excess
DEAD	diethyl azodicarboxylate
DHP	3,4-dihydro-2*H*-pyran
Dibal-H	diisobutylaluminium hydride
DMAP	4,4-dimethylaminopyridine
DMF	*N*,*N*'-dimethylformamide
DMSO	dimethylsulfoxide
EI	electron ionisation
ether	diethyl ether or ethoxy ethane
FAB	fast atom bombardment (xenon source); sample in 2-nitrobenzyl alcohol
GA	Gibberellin
h	hour(s)
HMPA	hexamethylphosphoramide (hexamethylphosphoric triamide)
HMPT	hexamethylphosphorous triamide
hplc	high performance (pressure) liquid chromatography
J	coupling constant (in Hertz)
LDA	lithium di-isopropylamide
Lindlar	(catalyst) palladium on calcium carbonate, poisoned with lead
Lit.	literature reference
m	multiplet
M^+ / $[MH]^+$	molecular ion / protonated molecular ion
*m*Cl*p*BA	3-chloroperoxybenzoic acid

MeOH	methanol (aka methyl alcohol or carbinol)
MOM	methoxymethyl chloride
mp	melting point
Ms	methanesulfonyl (mesyl)
MS	Mass spectroscopy
m/z	mass to charge ratio
NBS	N-bromosuccinimide
NMO	4-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
PCC / PDC	pyridinium chlorochromate / dichromate
petrol	petroleum ether (aka light petroleum) of the boiling range 40-60˚C
Ph	phenyl
PMB	*para*-methoxybenzyl
ppm	parts per million
PPTS	pyridinium *para*-toluenesulfonate
q	quartet
Rochelle Salt	(+) Sodium potassium tartrate
R_f	retention factor
s	singlet
SEM	2-(trimethylsilyl)ethoxymethyl
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilyl
Tf	trifluoromethanesulfonyl (triflyl)
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl or tetramethylsilane
TPAP	tetrapropylammonium perruthenate
Ts	4-toluenesulfonyl (tosyl)
δ	chemical shift (in ppm) from TMS
υ_{max}	IR frequency maxima (4000-500cm^{-1} range measured)
λ_{max}	UV frequency maxima (400-200nm^{-1} range measured)
18-c-6	18-crown-6 ether aka 1,4,7,10,13,16-hexaoxacyclooctadecane
Δ / Δ_R	Heated / heated to reflux

PARTIAL SYNTHESIS OF MYCINOLIDE III AND THE MYCINOIC ACIDS

CHAPTER ONE:

Introduction

1 Introduction

1.1 General Introduction to Antibiotics

There are 87 antimicrobials on the UK market for human use[1] which are classified as antibiotics, sulfonamides, quinolides, antimycobacterials and those of miscellaneous structure; of the antibiotics there are many sub-classes, those of greatest economic importance are penicillins and cephalosporins (and the related β-lactams and carbacephems), aminoglycosides, tetracyclins, quinolones and macrolides[2]. Antibiotics are defined as agents produced by living organisms which act in low concentration to inhibit the action of other organisms[3]; antibiotics are secondary metabolites (*i.e.* they do not appear to have an essential function for the producing organism).

The first of the macrolide antibiotics to be identified was picromycine in 1950, isolated from *Streptomyces fellens* (a soil bacteria). The macrolide was characterised as having an amino sugar attached to a 14 membered lactone with one degree of unsaturation[4]. Subsequently several similar structured antibiotics were recognised, and the term "macrolide" was used by Woodward to describe the new class of anti-infectives[5]; however, "macrolide" is now used to describe all medium to large ring lactones, thus antibiotics with at least one sugar attached to a multi-oxygenated partially unsaturated macrolide are called the polyoxomacrolides. Some examples of 12, 14 and 16-membered ring structures of polyoxomacrolide antibiotics are depicted in figure 1.1; rings devoid of the sugar moieties are called aglycones.

Of all the wide range of polyoxomacrolide antibiotics developed, it should be noted that only erythromycin (Erymax, Stiemycin®) and derivatives with modified sugar groups (e.g., Eryped, Erythrocin, Ilosone®), clarithromycin (Klaricid®) and azithromycin (Zithromax®) are currently licensed for human use in the UK. Spiramycin (Selectomycin, Rovamycin®) is used for antimicrobial vetinary practice and tylosin (Tylar®) is a growth promoter in animal feed[2]. The original polyoxomacrolide antibiotic available for clinical use was erythromycin, a 14-membered ring macrolide extracted from *Streptomycetes erythreus*, first used in 1951[6]. The other two licensed agents are semi-synthetic derivatives of erythromycin: azithromycin is an azalide, a 15-membered ring containing a nitrogen, whilst clarithromycin has a 6α-methoxy group

which prevents the 6-hydroxyl interacting with the 9-carbonyl to form a lactol, thereby preventing the initial degradation step of the ring as occurs *in vivo* with erythromycin. The newer polyoxomacrolides show less activity against Gram-positive and more against Gram-negative bacteria than erythromycin[7].

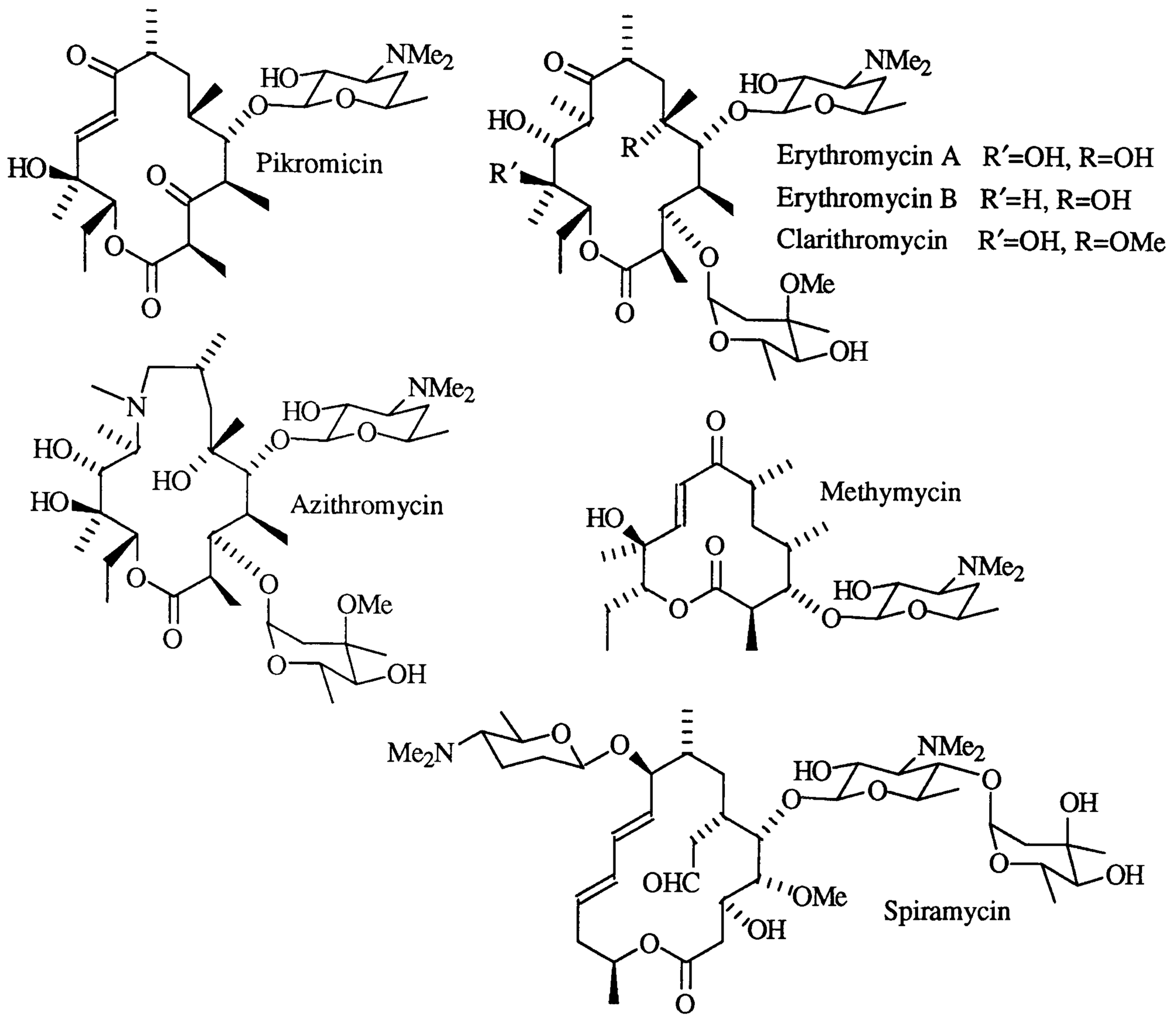
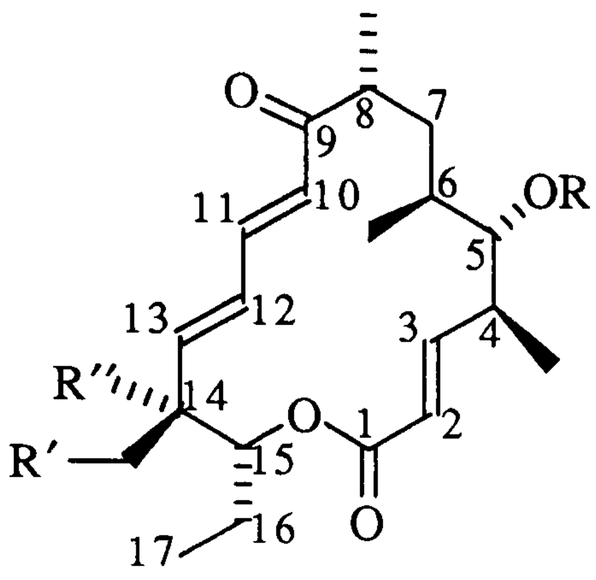


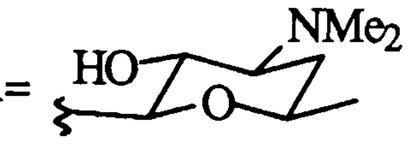
Figure 1.1: Examples of polyoxomacrolide antibiotics.

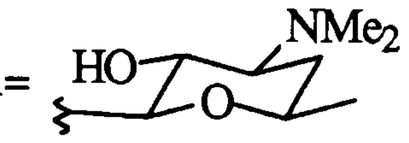
Mycinamicins are a class of polyoxomacrolides first isolated in 1980 from *Micromonospora griseorubida*, a soil actinomycete; these macrolides have a 16-membered ring aglycone ("mycinolide") with 5-O-β-D-desosaminylose and 17-O-β-D-mycinosylose sugar derivatives attached[8]. Mycinamicins have a higher activity against Gram positive bacteria than erythromycin, including against *Staphlococcus aureus* which has become a problem in hospitals by being resistant to a wide range of antibacterials[9]. Hence, the simultaneous application of both mycinamicins and currently licensed erythromycin derivatives in clinical practice may provide synergistic effects in eradication of bacterial infections.

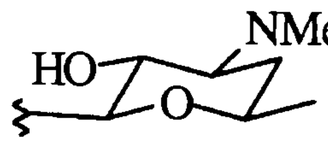
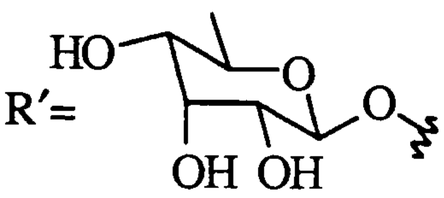
1.2 Biosynthesis of Mycinamicins

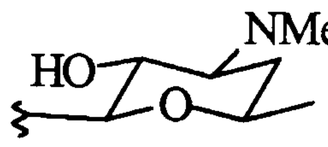
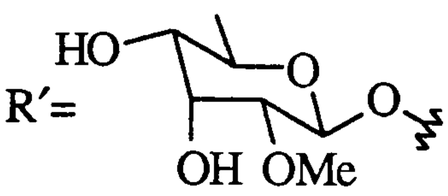


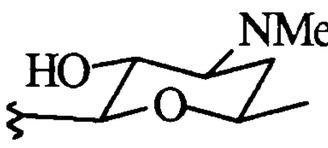
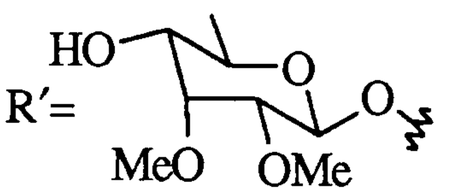
Protomycinolide IV **(1)** R,R′,R″=H

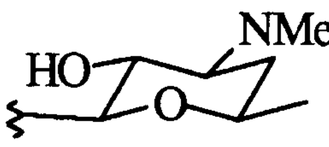
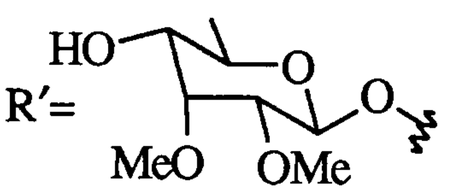
Mycinamicin VIII **(2)** R=  R′,R″=H

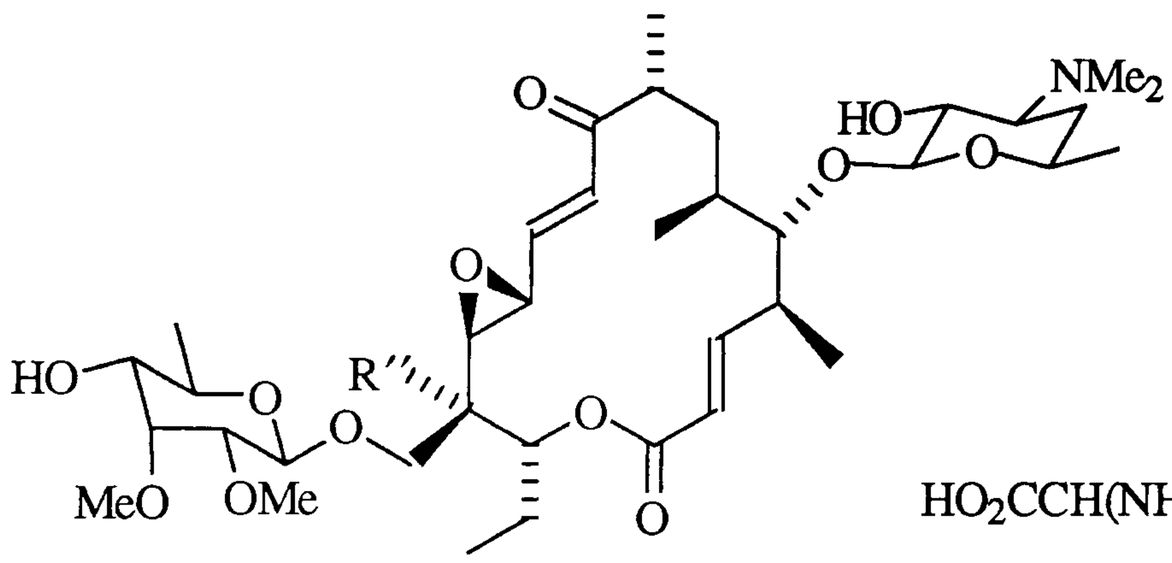
Mycinamicin VII **(3)** R=  R′=OH R″=H

Mycinamicin VI **(4)** R=  R′=  R″=H

Mycinamicin III **(5)** R=  R′=  R″=H

Mycinamicin IV **(6)** R=  R′=  R″=H

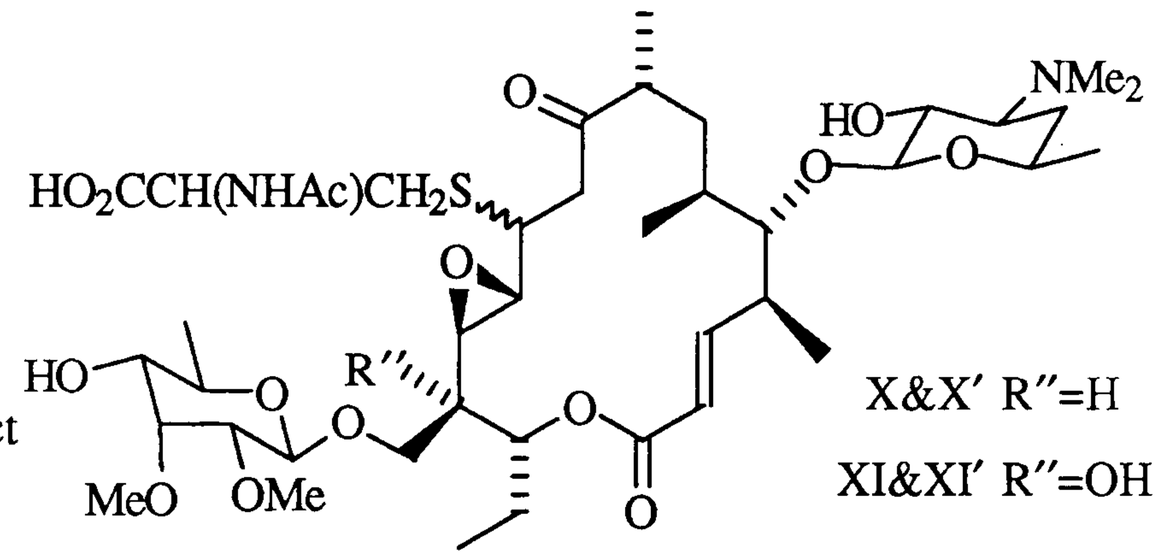
Mycinamicin V **(7)** R=  R′=  R″=OH



Mycinamicin I **(8)** R″=H

Mycinamicin II **(9)** R″=OH

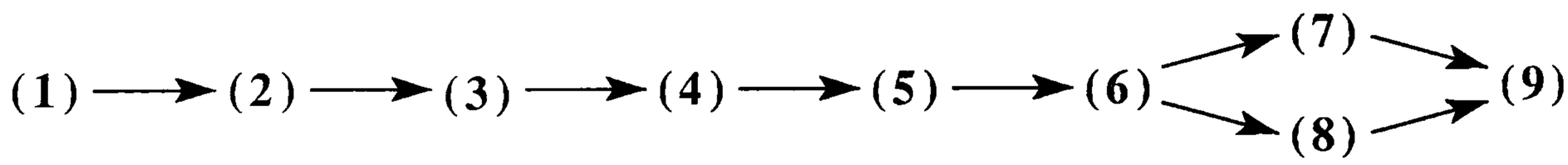
Minor by-product diastereomeric mycinamicins



X&X′ R″=H

XI&XI′ R″=OH

Figure 1.2: The isolated mycinamicin antibiotics[8].



Scheme 1.1: The pathway of mycinamicin biosynthesis in *M. griseorubida*[10].

Dextrose is the substrate for the biosynthesis of both the sugar groups in the mycinamicins, indeed in nearly all polyoxomacrolide antibiotics[11]. In an analogous

manner to fatty acid and polyketide biosyntheses, the aglycone is derived from acetyl CoA and propionyl CoA and the carboxylated derivatives malonyl CoA and methylmalonyl CoA respectively. The units are condensed *via* the thio-esters as a series of *pseudo*-Claisen condensation, reduction, elimination and reduction reactions, mediated by carboxylases, decarboxylases, NADPH and polyketide synthase[12].

By a combination of ^{13}C and ^{14}C labelling studies plus ^{1}H and ^{13}C NMR spectra it has been shown that the aglycone is constructed[13] as shown in figure 1.3. Use of ^{18}O demonstrated that the esterification to form the lactone is the final step in the biosynthesis of the aglycone, followed by glycosidation and modification of the sugars[14]. The 11ζ-*N*-acetylcysteamine derivatives (mycinamicins X, X′, XI and XI′) are by-products of the biosynthesis with far weaker biological activity than mycinamicin I or II and are produced only in low quantities[15].

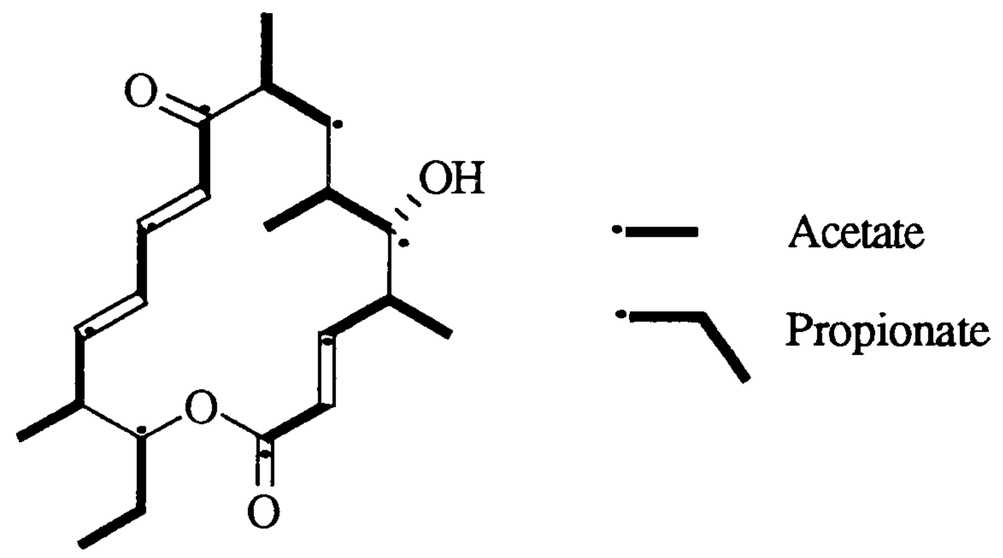
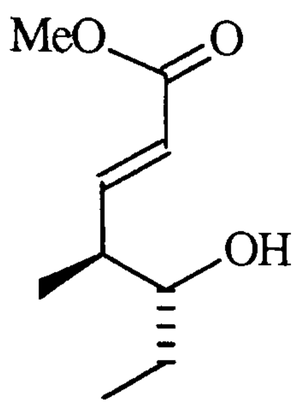


Figure 1.3: Biosynthetic units position in protomycinolide IV (**1**).

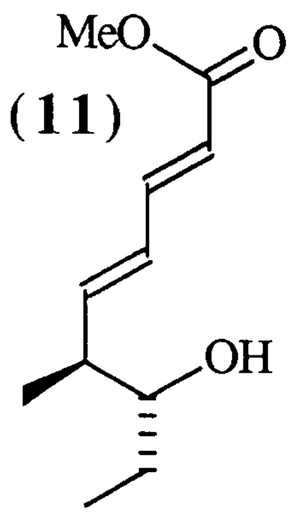
Only in the case of the mycinamicins[16] have coherent derivatives of the intermediates which form the macrocycle been isolated. The methyl esters of these have been isolated and characterised from the culture broth of *M. griseorubida* (figure 1.4). Similar compounds[17] were also found from the culture of mycinolide-forming *Streptomyces spp*. The isolation of these intermediates indicates a chain construction process consistent with polyketide biosynthesis adding β-ketones and modifying at each step before the next unit is added[12]. The seco-acid was also isolated, which was the first seco-acid to be isolated from a fermentation culture[18]. The protomycinolide IV (**1**) is the final precursor before glycosidation to form mycinamicin VIII. A ^{13}C thioester derivative of mycinoic acid I was incorporated into mycinolide IV by biosynthesis[19], further supporting the theory that the chain elongation process proceeds systematically

to form mycinolides; a similar continual extension process occurs in the fungal production of erythromycin[20].



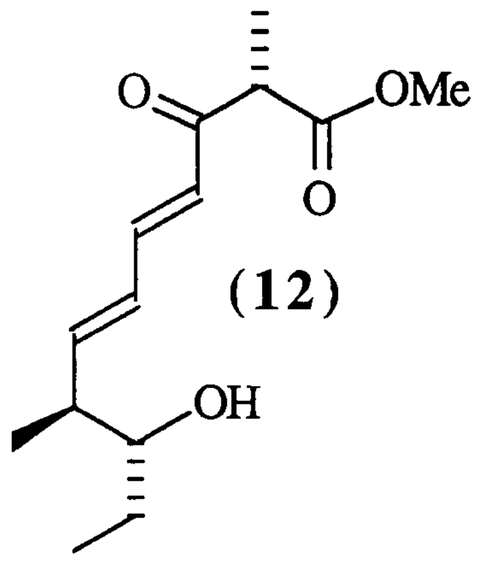
(10)

Mycinoic acid
I methyl ester



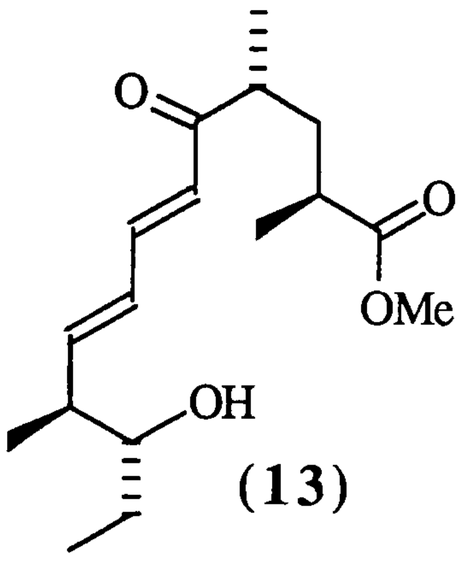
(11)

Mycinoic acid
II methyl ester



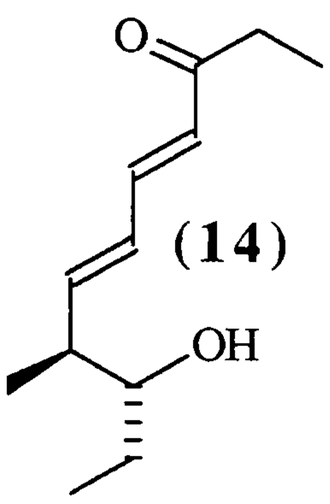
(12)

Mycinoic acid
III methyl ester



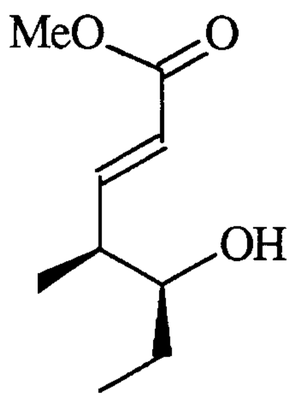
(13)

Mycinoic acid
IV methyl ester



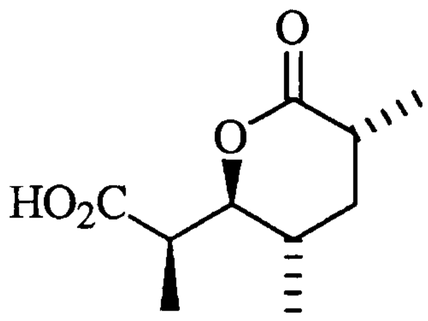
(14)

Decarboxy-
mycinoic
acid III



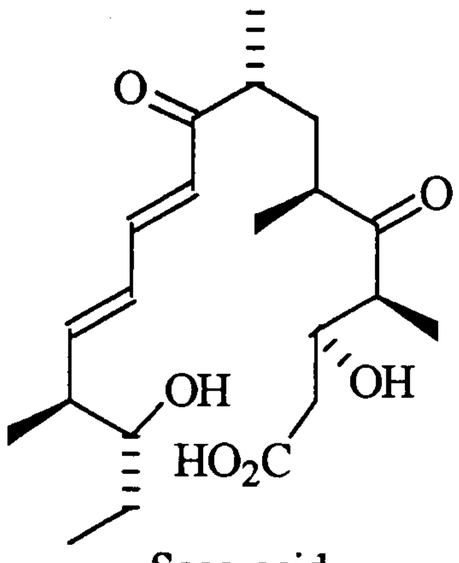
(15)

Epimycinoic
acid I
methyl ester



(16)

Prelog-Djerassi
lactonic acid



Seco-acid,
precursor to
protomycinolide IV

Figure 1.4: Products of biosynthetic route to the aglycone of mycinamicins.

Fermentation of 30 litres of a microbial culture of *M. griseorubida* , followed by extraction with organic solvents and evaporation gave a crude powder (5.2g) which contained a variety of secondary metabolites, which was purified by column chromatography to give only 0.15g of mycinamicin III[8]. Extensive analysis of the anabolic pathways of mutant strains unable to produce mycinamicins by Hayashi *et al.* has indicated the pathway of biosynthesis of the macrolide ring[18].

The methyl esters of mycinoic acids I **(10)** and II **(11)** plus epimycinoic acid I **(15)** and decarboxy-mycinoic acid III **(14)** have been synthesised by Takano and co-workers[21] using an acyclic precursor (section 1.5). These matched the natural methyl esters found by Kinoshita *et al.*[22].

Hence, both the aglycone and the mycinoic acid's total synthesis were the target of the project to enable a greater supply of the mycinolides, which may be glycosidated to give the mycinamicins for biological assay. Whilst the aglycone common to

mycinamicins III, IV, VI and VII was the target of the project, it should be noted that there is a total absence of antibiotic activity by the aglycone alone[23].

1.3 Total Synthesis of Polyoxomacrolides

The total synthesis of the major classes of antibiotics then in medicinal use, (the penicillins, cephalosporins, aminoglycosides and tetracyclins) had been completed by the late 1960's. However, as all had proved to be more effectively and quickly produced by fermentation technology or semi-synthesis based on a natural building block, none were made commercially by total synthesis. This trend has continued to date with the exception of the comparatively simple aromatic antibiotic chloramphenicol[2]. The only major group of antibiotics in clinical practice in the 1960's without a total synthesis of a sample compound was the macrolides, which posed such a serious challenge to chemical formation that the Nobel Prize winner Woodward had written "erythromycin, with all our advantages, looks at present quite hopelessly complex, particularly in view of of its plethora of asymmetric centers..."[24].

The three main problems that required resolution were:

(i) Control of both regio- and stereo-addition of substituents to the proto-aglycone;

(ii) the selective esterification of the desired alcohol functionality with the carboxylic acid to form the macrocylic aglycone[25];

(iii) glycosidation has to date been achieved with few polyoxomacrolides, and commercial semi-synthetic processes utilize the fragments with the sugars already attached whilst the ring of the antibiotic is manipulated[26].

The first polyoxomacrolide to be completed by total synthesis was methymycin in 1975, which showed that a seco-acid could be induced to cyclise to the chosen lactone, but a requirement is that the favoured low energy conformation must be achieved in order to obtain the correct lactone. The macrocyclisation often requires a high dilution factor for effectiveness; also the creation of the asymmetric centres on the seco-acid precursor was not facile[26]. Erythromycin A was synthesised by Woodward *et al.* in 1981[27], and both carbomycin B and tylosin by Kinoshita and Tatsuta[28,29];

however, these remain as some of the few total syntheses of the polyoxomacrolide antibiotics, due to the difficulty of glycosidation.

Four general pathways to forming the aglycones have been utilized[23]:

(a) The *ring* approach, which makes use of conventional α and β positions on a ring carbon as prochiral centres and of stereofacial attack upon the ring, which allows the retention of chirality on ring cleavage. Examples from such a pathway include methymycin[26] (12-membered ring), erythromycin A[27] and 3 routes to erythronolides[30] (14-membered rings) and the tylonide synthesis by Greico[31] (16-membered ring).

(b) The *carbohydrate* approach uses the inherent stereochemistry and functionality of sugars, which may be transferred to the products; the benefits include utilizing a cheap and homochiral starting material. If the enantiomer of a macrolide is desired for antimicrobial trials, the (*L*) (unnatural) sugar may be used with identical methodology; biosynthesis will often not allow such a route due to chirally specific enzymes. Whilst no syntheses of 12-membered polyoxomacrolides have used this approach, it has been used in both the syntheses of erythronolide A[32] (14-membered ring) and tyolonide and carbomycin B by Kinoshita and Tatsuta[28,29] and Nicolaou[33] (16-membered rings).

(c) The *acyclic* approach utilizes stereoselective construction by treatment of an achiral starting material with chiral reagents to form chiral fragments. Other methods of stereocontrol that have been applied are chiral derivatisation agents or chiral chromatography[25]. Examples of products from such a route include methylnolide (12-membered ring)[34], narbonolide[35] (14-membered ring) and the tyolonide synthesis by Masamune (16-membered ring)[36].

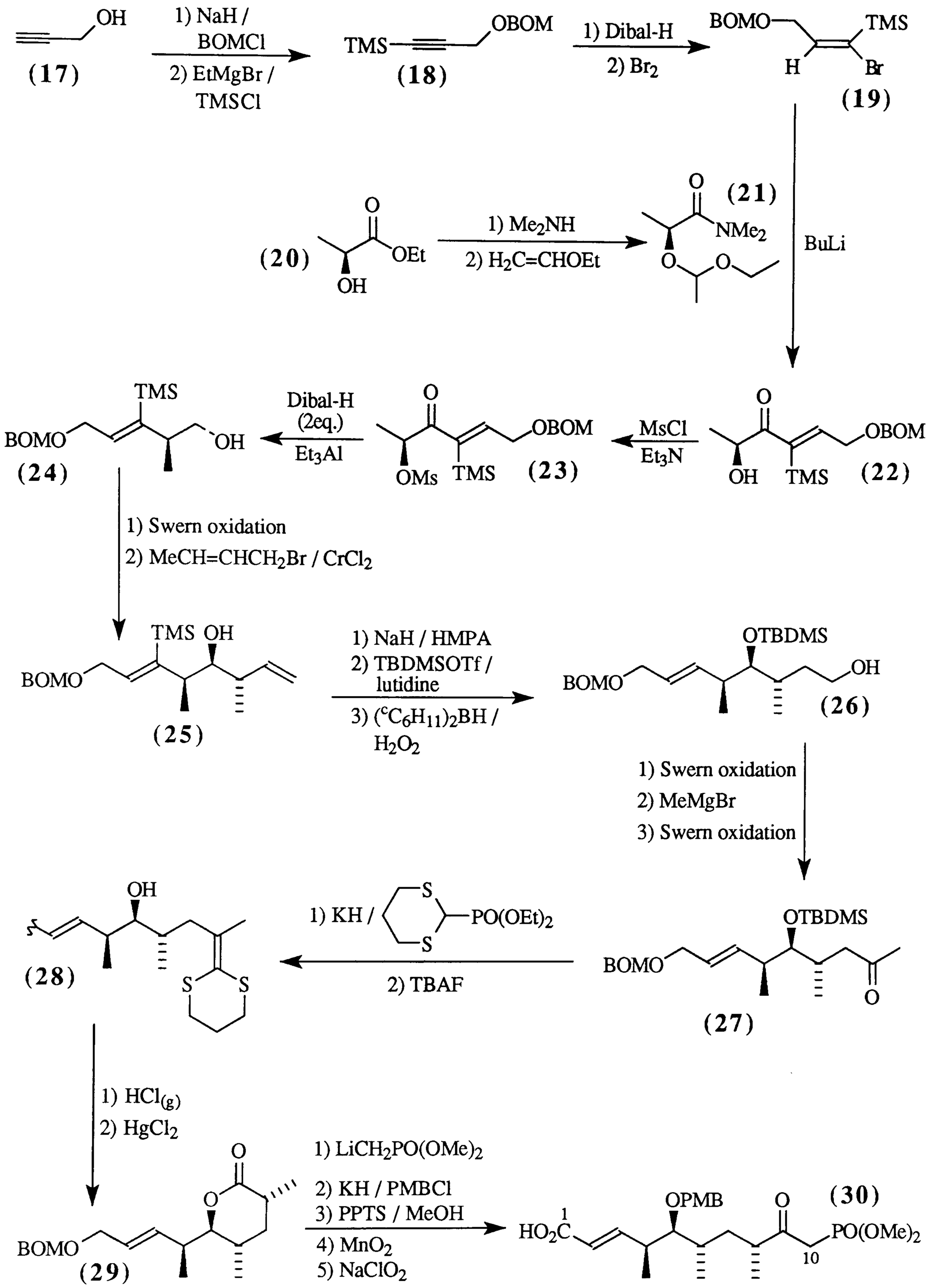
(d) The *macrocyclic* approach of controlling reactions *via* an intact large ring with pre-existing asymmetric centres to induce further diastereoselecive functionalisation. This route has been utilized far less than the previous three routes, largely due to the requirement of pre-formation of a ring from partially derivatised components, which themselves are not simple, e.g., the total construction of racemic deoxyrosaranolide hemiacetal utilized the aldehyde derivative of mycinoic acid as a construction unit for the aglycone[37].

The first three approaches require a cyclisation reaction to form the aglycone, but as the cyclisation of such large rings is generally disfavoured and requires an intermediate that can assume the low energy and diamond lattice shape of the final aglycone, this is often a problem stage in the formation of the macrolide[38]. Consequently, much research has gone into solving the problem of cyclizing intermediates that do not favour the desired products[25].

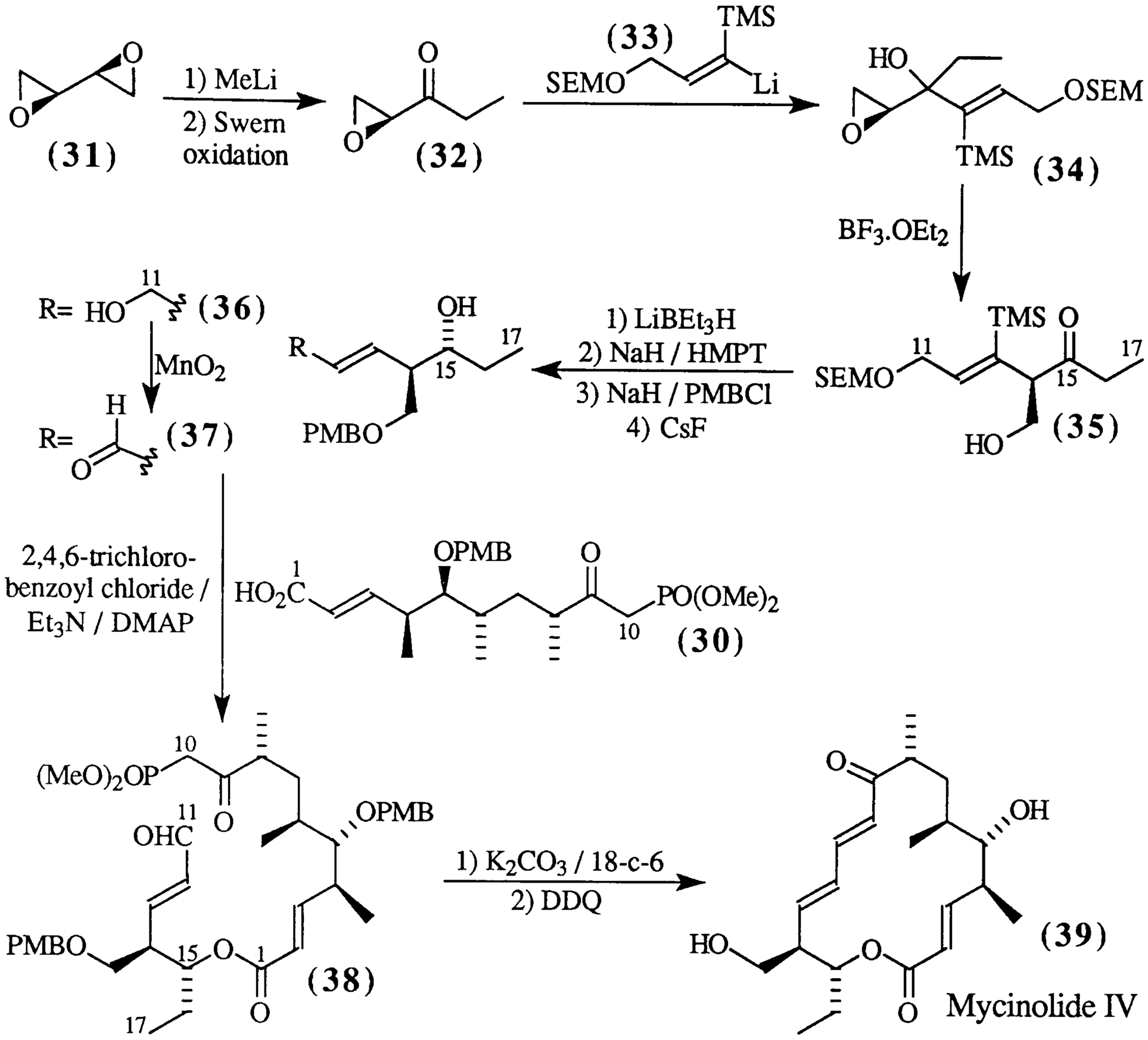
1.4 Previous Syntheses of Mycinamicins and Mycinolides

Mycinamicins have been subject to only one total synthesis[39], by Suzuki in 1988[40], but two aglycones have been completed, plus several syntheses of fragments. As one of the proposed targets was the aglycone common to mycinamicins III, IV, VI and VII, the previous syntheses will be briefly outlined.

The mycinolide IV synthesis by Suzuki[41,42] using an acyclic approach gave an overall yield of only 1.8% in 28 steps from propargyl alcohol **(17)** (scheme 1.2) and the bis-epoxide **(31)** (scheme 1.3), derived from (*S*) lactate and (*L*) tartrate respectively, but required resolution. Interesting features of the synthesis include the triethylaluminium catalysed rearrangement of the secondary alcohol **(22)** via the methansulfonate - aluminium alkoxide intermediate **(23)** to the primary alcohol **(24)**, with total stereocontrol. The subsequent aldehyde reacted with chromyl chloride and crotyl bromide forming two new asymmetric centres. Asymmetric control was also obtained upon reaction of **(28)** with dry $HCl_{(g)}$ and dithioketal removal with mercuric chloride to form **(29)**. The *bis*-epoxide derived alcohol **(34)** was also rearranged by a Lewis acid again with no isomerisation to form **(35)**. Macrocyclisation of **(30)** and **(37)** was achieved in 21% yield over the two steps, and the protective groups oxidatively cleaved to give mycinolide IV. The addition of the sugar residues was achieved utilizing a novel modified Koenigs-Knorr glycosidation using fluorinated glycosyl groups activated by Cp_2ZrCl_2-$AgClO_4$[43]; initially β-D-desosaminose was added to the aglycone giving mycinamicin VII **(3)** in 72% yield, then β-D-5,6-dimethoxy-mycinose was attached to form mycinamicin IV **(6)** in 86% yield.

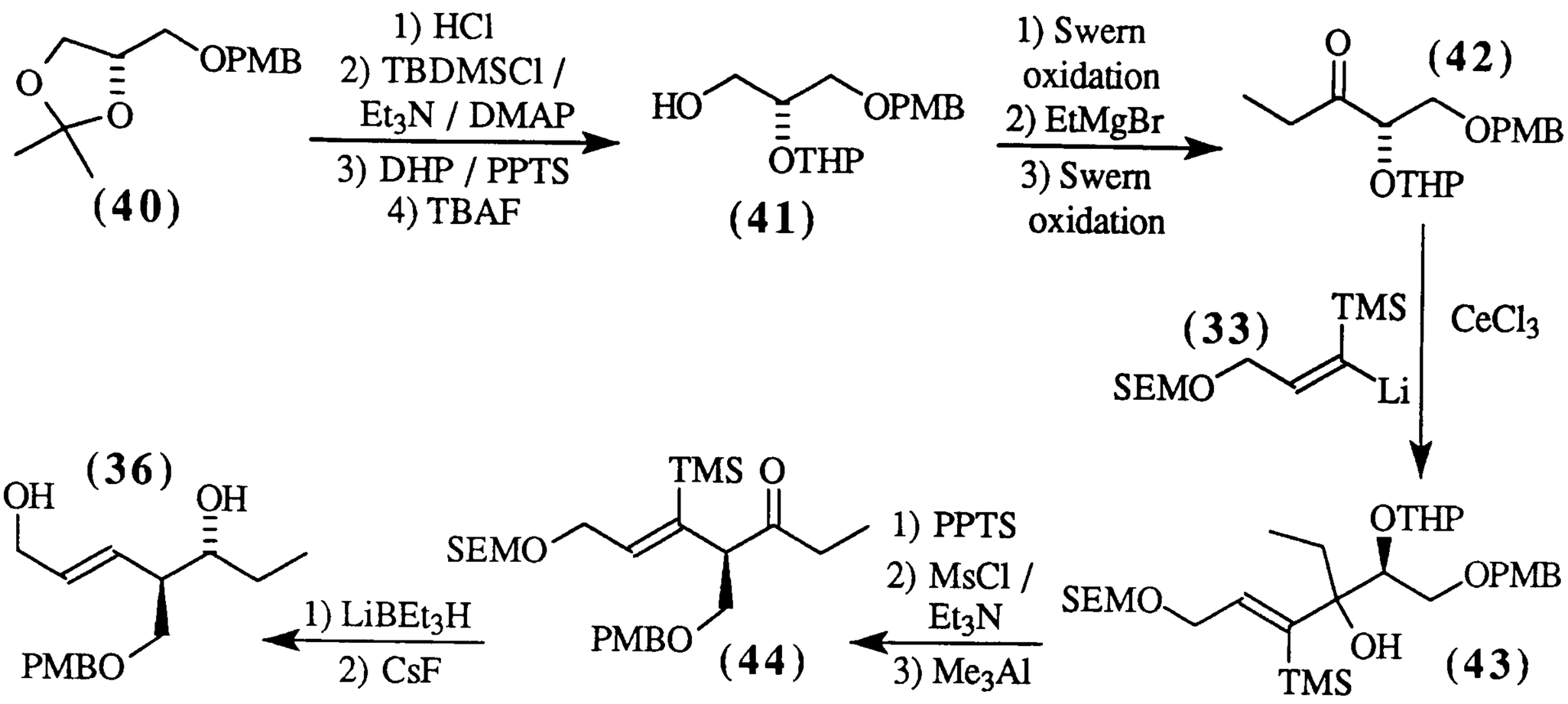


Scheme 1.2: Suzuki synthesis of fragment C_1-C_{10} of mycinolide IV.



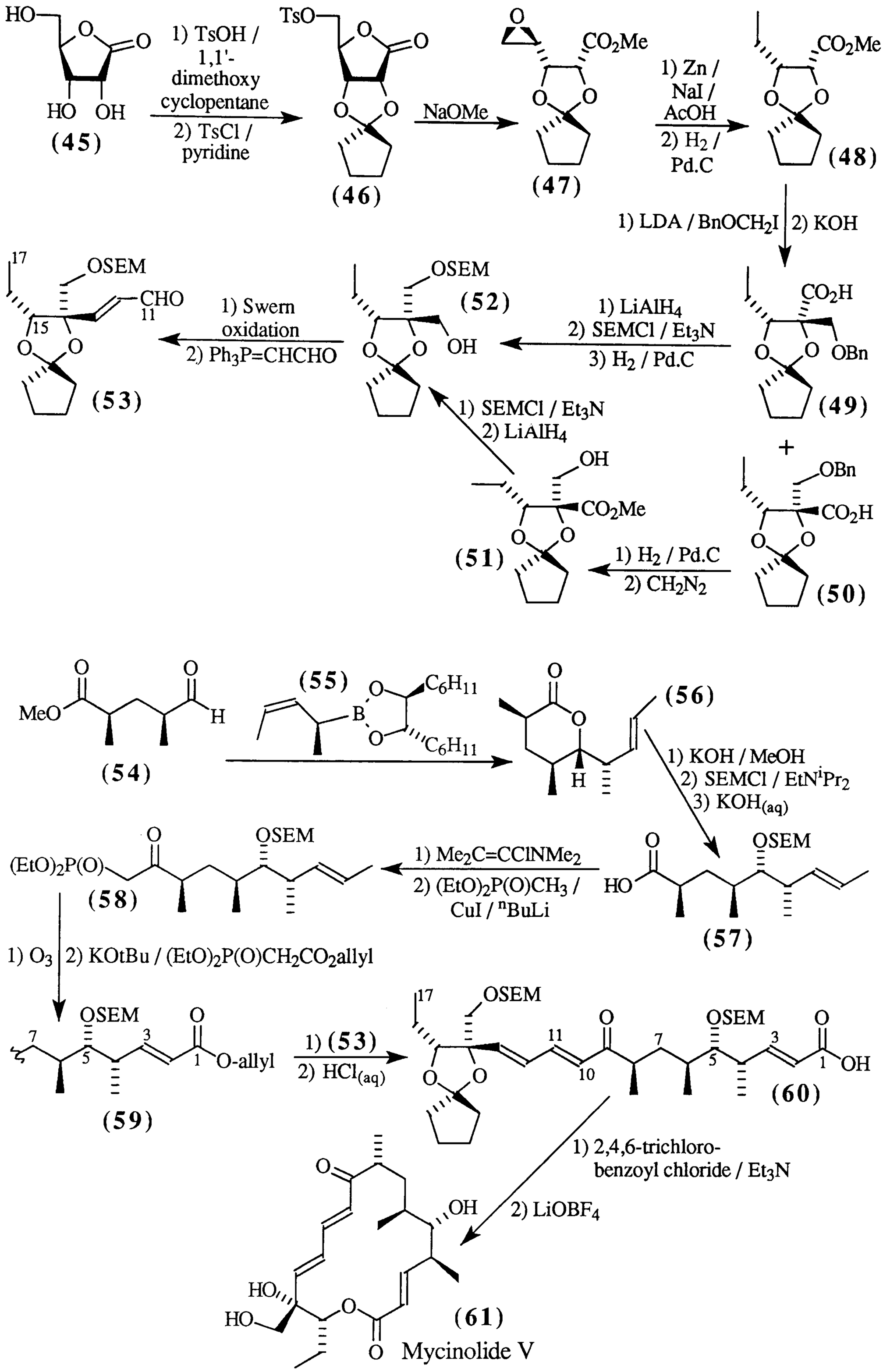
Scheme 1.3: Suzuki synthesis of fragment C_{11}-C_{17} and cyclisation to mycinolide IV.

Suzuki and co-workers improved dramatically the yield of the aldehyde **(37)** by use of a *ring* approach to forming the carbon skeleton, using a chiral glycerin derivative **(40)** (scheme 1.4)[44]; the initial route using the *bis*-epoxide **(31)** (scheme 1.3) gave a yield of **(36)** of only 13% over 8 steps, but use of **(40)** returned the allyl alcohol **(36)** in 40% over 13 steps. The dioxolane **(40)** was opened, selectively protected and deprotected to give the primary alcohol **(41)** which was oxidised to an aldehyde; an ethyl group was added to the ketone to give the secondary alcohol followed by a second Swern oxidation to give **(42)**. Further oxidation and cerous chloride mediated coupling of the organolithium **(33)** with **(42)** gave the alcohol **(43)** in 95% yield. The alcohol **(43)** was rearranged and modified in a similar manner to the original route to obtain the allylic alcohol **(36)**, which was subsequently oxidised to the conjugated aldehyde **(37)** for coupling to the C(1)-C(10) fragment **(30)** (scheme 1.3).



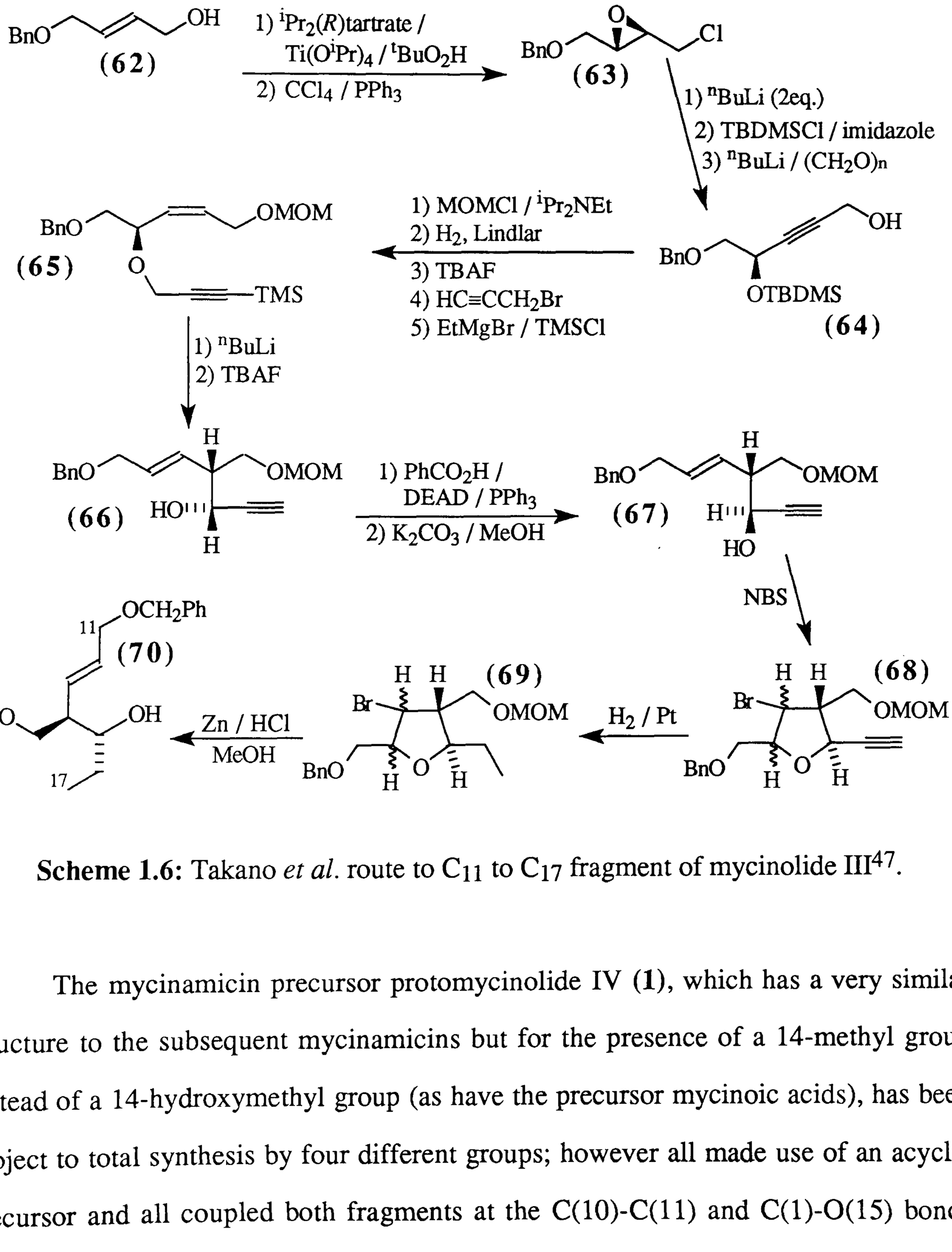
Scheme 1.4: Modified route by Suzuki to form C_{11}-C_{17} fragment of mycinolide IV.

Hoffman's synthesis of mycinolide V was based upon a chiral starting material in a combined acyclic and carbohydrate approach, utilising only one of the three asymmetric centres in D-ribonolactone (**45**)[45] (scheme 1.5). A summary of the route includes the complex divergence and convergence stages of converting the ester (**48**) to the partially protected diol (**52**); the use of LDA with benzyliodomethyl ether as electrophile epimerised the carbon α to the carbonyl, and on saponification gave a 3:2 mixture of the two esters (**49**) and (**50**). Saponification with aqueous potassium hydroxide and use of cyclohexylamine enabled preferential crystallisation of the more prevalent salt (**49**) from the mother liquors. Two differing routes allowed (**52**) to be synthesised from either ester, which was further oxidised to the conjugated aldehyde (**53**). The 'Masamune aldehyde' (**54**)[46] (an intermediate in the total synthesis of tylonide by the Masamune group) was reduced with concurrent high asymmetric induction by the chiral *Z*-pentenylboranate (**55**) to give the δ-lactone (**56**) in 80% yield and 94% d.e. The overall number of steps to obtain the aglycone (**61**) was only 21 in an overall yield of 2.7% (scheme 1.6).



Scheme 1.5: Hoffman route to the aglycone of mycinamicin V[45].

The synthesis of fragment C_{11} to C_{17} of mycinolide III by Takano was based upon a Sharpless epoxidation to induce chirality into an acyclic starting material (**62**)[47] (scheme 1.6). The [2,3]-Wittig rearrangement of (**65**) gave the alcohol (**66**) as the only product in 97% and generated a second stereo-centre. The fragment (**70**) was made in 12% overall yield in 18 steps; and the ingenious use of the temporary tetrahydrofuran moiety in (**68**) and (**69**) avoided any hydrogenation of the double bond, but enabled reduction of the alkyne. However, the molecule could not be extended further to form more of the mycinolide.



Scheme 1.6: Takano *et al.* route to C_{11} to C_{17} fragment of mycinolide III[47].

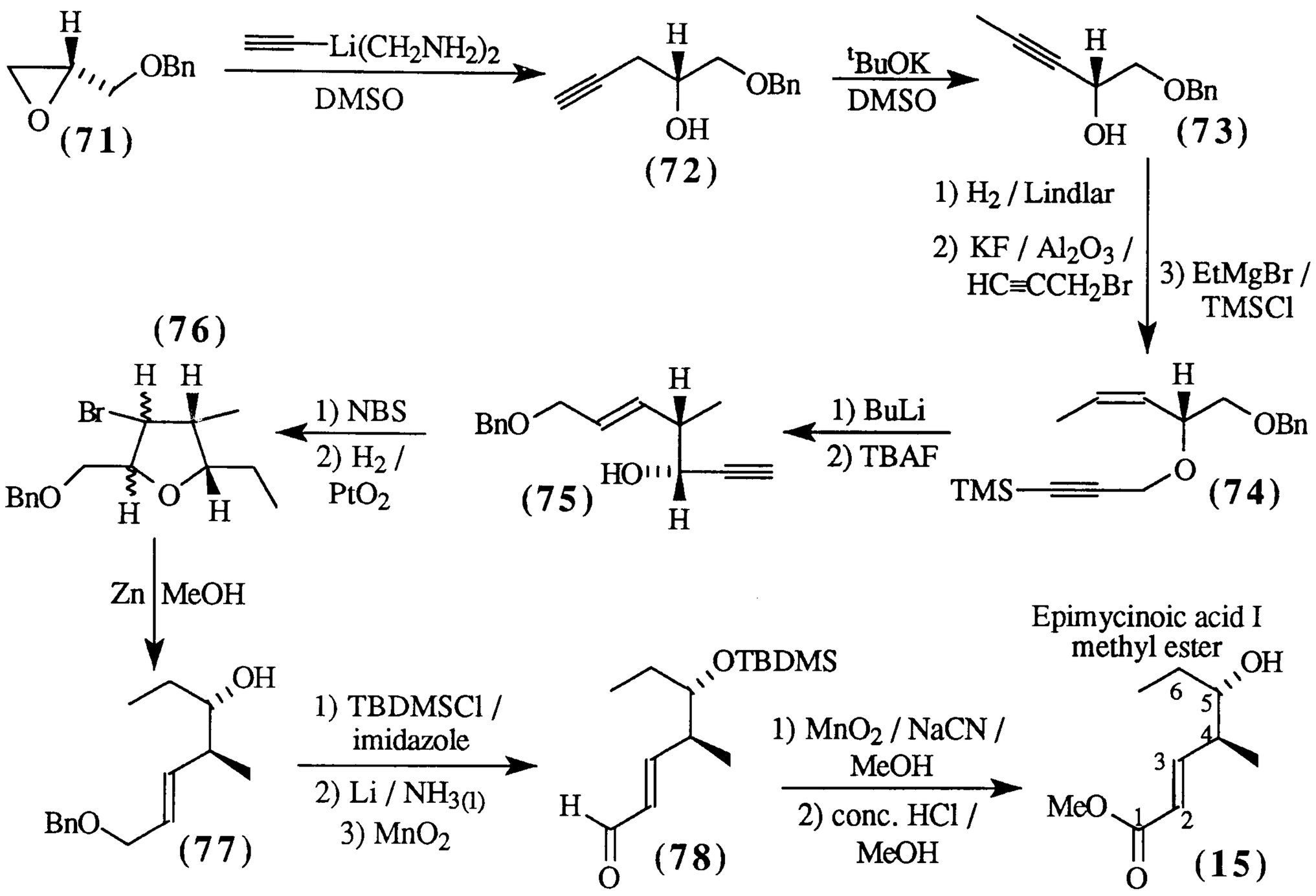
The mycinamicin precursor protomycinolide IV (**1**), which has a very similar structure to the subsequent mycinamicins but for the presence of a 14-methyl group instead of a 14-hydroxymethyl group (as have the precursor mycinoic acids), has been subject to total synthesis by four different groups; however all made use of an acyclic precursor and all coupled both fragments at the C(10)-C(11) and C(1)-O(15) bonds (figure 1.2).

Protomycinolide IV was first synthesized by Yamaguchi and co-workers in 1984[48] utilizing *meso*-2,4-dimethylglutaric anhydride and propargyl alcohol as the initial substrates for synthesis, both of which are achiral; the macrolide was obtained in 33 steps and in 0.38% yield, although the final stage to enable macrocyclisation was acheived in only 53% yield; however, a new route to Prelog-Djerassi lactonic acid **(16)** was also evolved. Suzuki *et al.*[42] (1986) used a very similar pathway to that developed for synthesis of mycinolide IV[41], but required 46 steps for 0.92% conversion of ethyl lactate derivatives to protomycinolide IV. The Takano group utilized homologous techniques to those used in the synthesis of the fragment C_{11}-C_{17}[47], and with further modifications gave the methyl esters of mycinoic acids I **(10)** and II **(11)**, epimycinoic acid I **(15)**, and decarboxymycinoic III also **(14)**[21]; protomycinolide IV **(1)** was obtained in 2.2% yield over 26 steps, including use of just one chiral building block as the basis for production of both the methyl esters of mycinoic acids and protomycinolide IV, demonstrating useful transfer of methodology. The last total synthesis of protomycinolide IV was developed in 1992 by Miyashita and co-workers[49], utilizing a technique based upon stereocontrolled opening of epoxy acrylates with trimethylaluminium, completing the synthesis in only 23 steps in 8% yield; a similar route using a Lewis acid rearrangement in a fundamental step gave a novel procedure to obtain Prelog-Djerassi lactonic acid **(16)**.

1.5 Previous Synthesis of Mycinoic Acids

The methyl esters of mycinoic acids I and II, epimycinoic acid I and decarboxymycinoic have been synthesised by Takano *et al.*[21] (figure 1.7); this is the only chemical synthesis of the polyketide intermediates of the mycinolide aglycone. *S*-(*O*)-benzylglycidol **(71)** was opened with the lithium alkyne to give the terminal alkyne **(72)** which was rearranged by base to an internal alkyne **(73)**. Partial reduction of the acetylene, a modified Williamson ether coupling and protection of the terminal acetylene with a TMS group gave **(74)**. A Wittig rearrangement of the allyl alcohol followed by removal of the silyl functionality gave **(75)**, which was used in the synthesis of all four mycinoic acid derivatives (schemes 1.7 and 1.8). In a series of

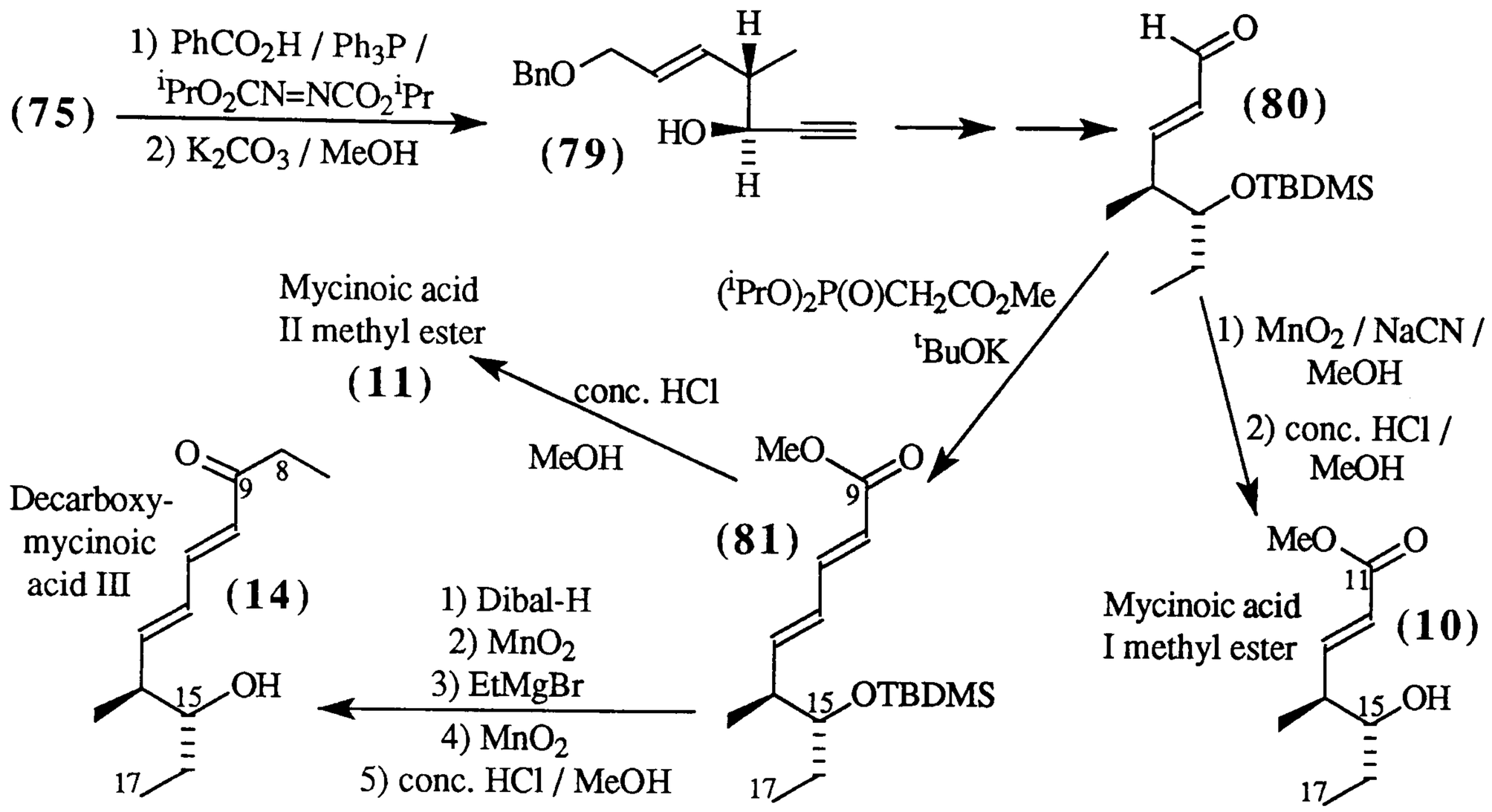
modifications that were very similar to those used in the synthesis of fragment C_{11} to C_{17} of mycinolide III (scheme 1.6)[47], the methyl ester of epimycinoic acid was synthesised by formation of a bromo ether, the alkyne hydrogenated and by use of zinc powder in hydrochloric acid the olefin regenerated (**77**). Protection of the secondary alcohol, followed by sequential deprotection, oxidation *via* (**78**) and methylation of the allylic alcohol gave a silyl ester, which was de-silated with mineral acid to give a product (**15**), that was identical to that of natural methyl epimycinoate I (figure 1.4) by spectral and physical data.



Scheme 1.7: Takano route to methyl epimycinoate I[21].

Inversion of the tertiary alcohol of (**75**) by a Mitsunobu reaction gave (**79**) for use as a common intermediate for the synthesis of decarboxymycinoic acid III and the methyl esters of mycinoic acids I and II (scheme 1.8). Identical treatment of (**79**) to that used upon the diastereomer (**75**) to derive (**78**) (scheme 1.7), gave the conjugated ketone (**80**). Oxidation, methylation and removal of the silyl protecting group gave methyl mycinoate I (**10**). Treatment of (**80**) with a Horner-Emmons reagent yielded the intermediate (**81**), which was de-silylated with acid to give methyl mycinoate II (**11**). Dibal-H reduction of the methyl ester of (**81**) to an allylic alcohol and oxidation to the

aldehyde enabled addition of an ethyl group *via* a Grignard reaction, the resultant allyl alcohol was again oxidised and the TBDMS group removed to yield decarboxymycinoic acid III (**14**). Thus four of the isolated intermediate mycinoic acid precursors to the aglycone ring of protomycinolide IV (**1**) had been formed.



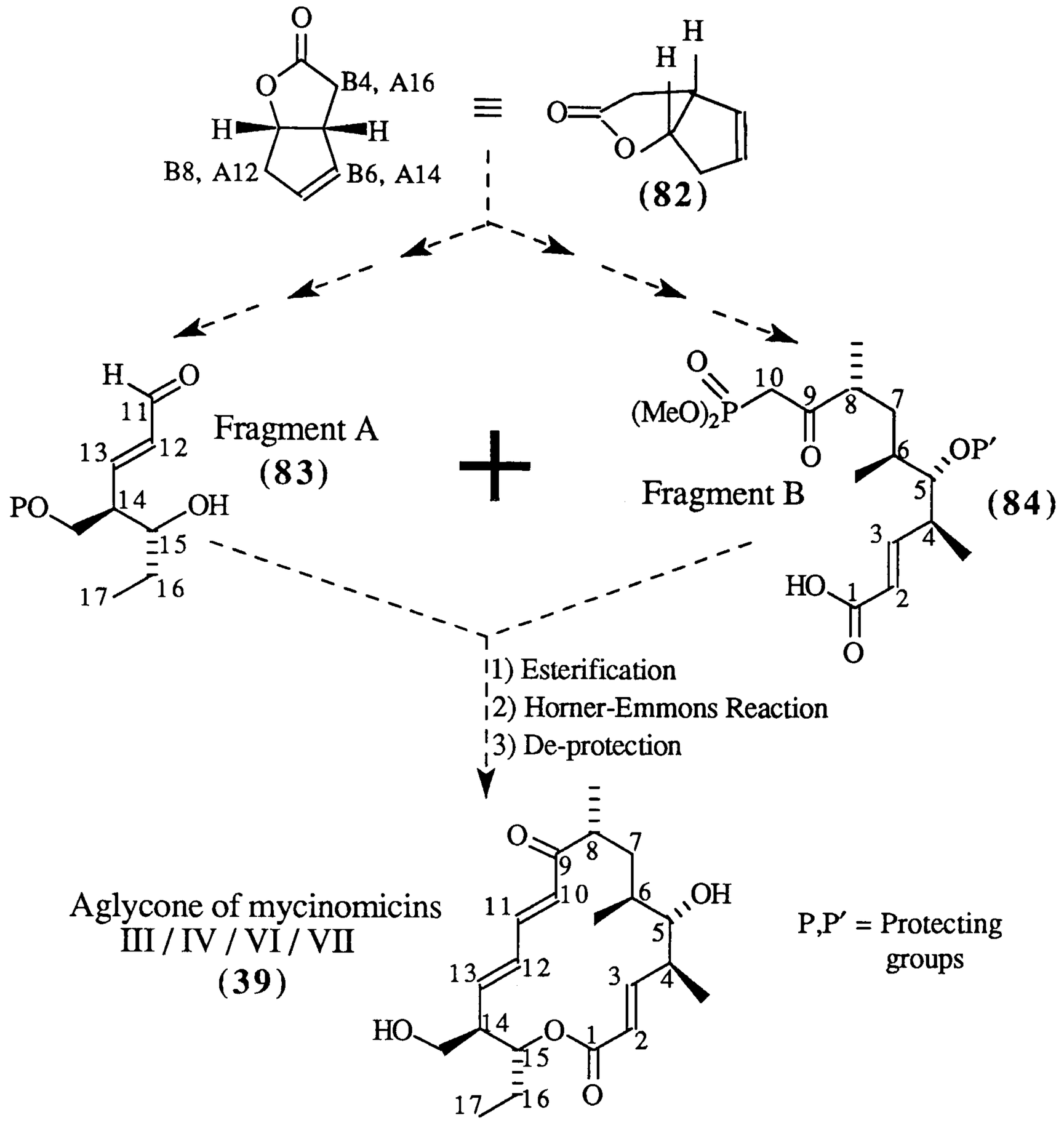
Scheme 1.8: Takano route to methyl mycinoates I, II & decarboxymyinonic acid III[21].

1.6 Proposed Route to the Synthesis of Mycinolide III

There have only been two reported total syntheses of mycinolides, that of the complete antibiotic mycinomicin IV by Suzuki *et al.*[40,43] and mycinolide V by Hoffman[45]. Numerous other polyoxomacrolides have been isolated and characterised (figure 1.1) and our aim was to develop a flexible approach to the total synthesis of a range of mycinolides. As detailed in section 1.2, usually during the biosynthesis of polyoxomacrolides the intact macrocycle is formed prior to release from the intracellular polyketide synthase. However, the mycinoic acids, the putative biosynthetic precursors to the mycinolides, have been isolated from the culture media. of *M. griseorubida*[22] (figure 1.4). Thus the proposed targets of synthesis were the aglycone of mycinamicin IV (**39**) (which is identical to the ring of micinamicin's III, VI and VII) and the mycinoic acids I and II.

Lactone (**82**) has been used extensively as the starting material for the synthesis of natural products, *e.g.*, prostaglandins and desoxyprostaglandins (prostaglandin

antagonists)[50], Prelog-Djerassi lactonic acid **(16)**[23,51], *cis*-Jasmone[52] and is currently under investigation in our group in the total synthesis of pseudomonic acids[53].



Scheme 1.9: Overview of proposed route to the aglycone.

The lactone **(82)** was chosen as the basis for the syntheses for several reasons.

(a) Development of a total synthesis of <u>both</u> the aglycone and the inherently closely related mycinoic acids *via* a convergent approach from common starting material **(82)** would allow the transfer of synthetic methodology between routes.

(b) The lactone is readily prepared in homochiral form (section 1.7). Homochiral (-) lactone **(85)** may be used as the basis to make the correct enantiomer of each product once synthetic methodology is optimised.

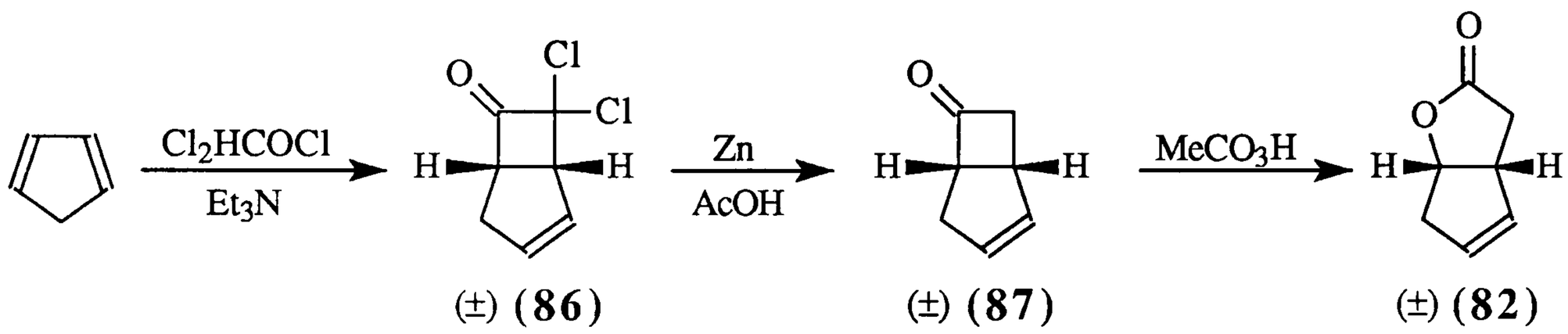
(c) The previous syntheses of mycinolides had not made full use of chiral centres present in the starting material (Hoffman[45]), or had utilized achiral substrates

(Suzuki[41,42], Takano[47]), whilst none had used the *ring* approach proposed in our strategy. The lactone (**82**) confers effective regio- and stereo- control due to the bridged nature of the bicycle.

(d) Synthesis of unnatural or isotopically labelled mycinoic acids and incubation with mutant strains of bacteria (that have partially blocked biosynthetic pathways, preventing synthesis of mycinoic acids) may enable the enforced uptake of such unnatural mycinoic acids, to allow production of novel antibiotics.

(e) The closely related intermediate protomycinolide IV (**1**) might be made by a very similar route to that to form mycinolide III, and any desired variations in structure installed by chemical means. All four previous routes to protomycinolide IV used an acyclic starting material, none had used macrocyclic, carbohydrate or ring approaches, thus new methods may be generated by this route.

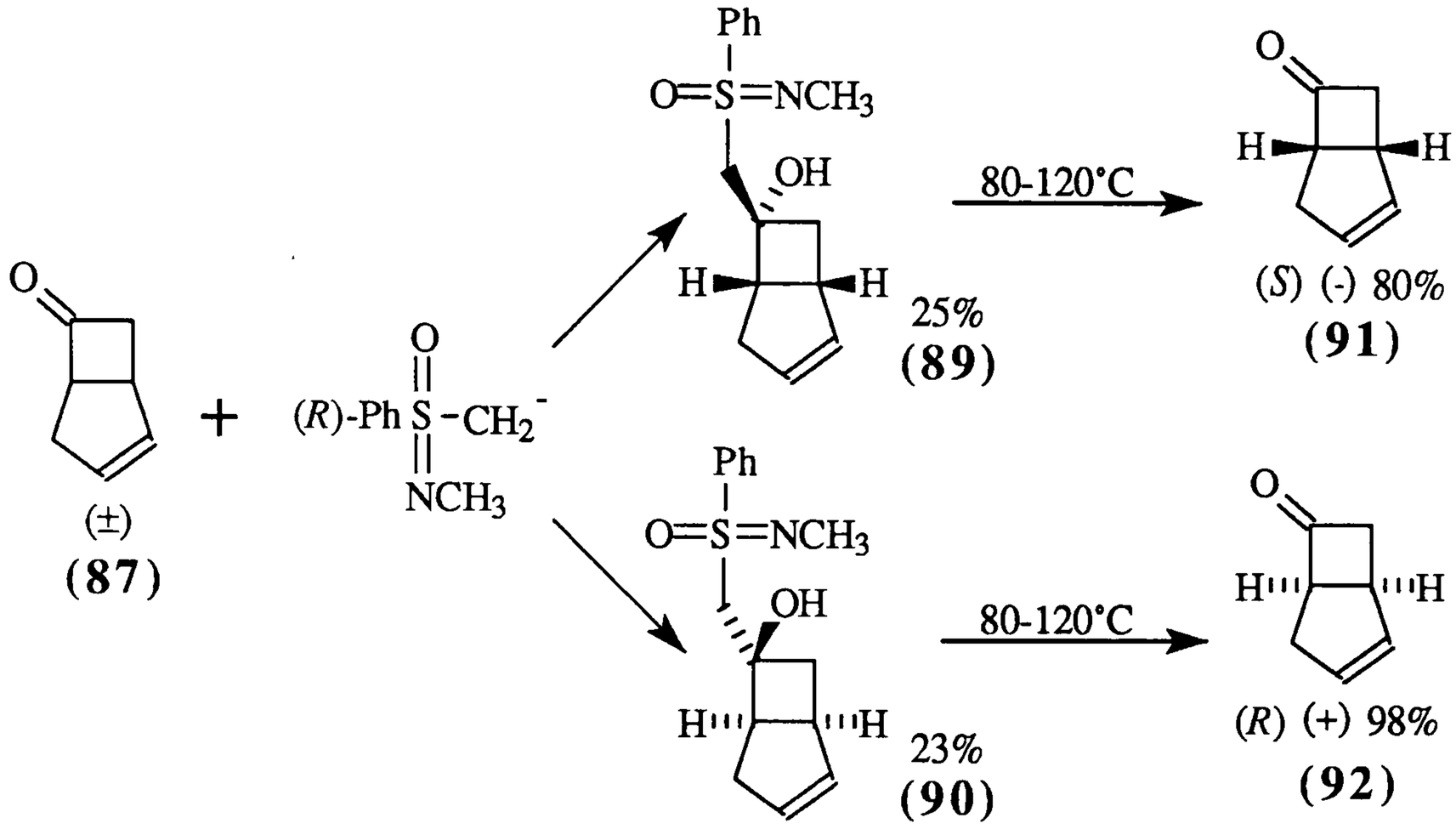
1.7 Syntheses of Homochiral Lactone



Scheme 1.10: Route to racemic lactone (**82**).

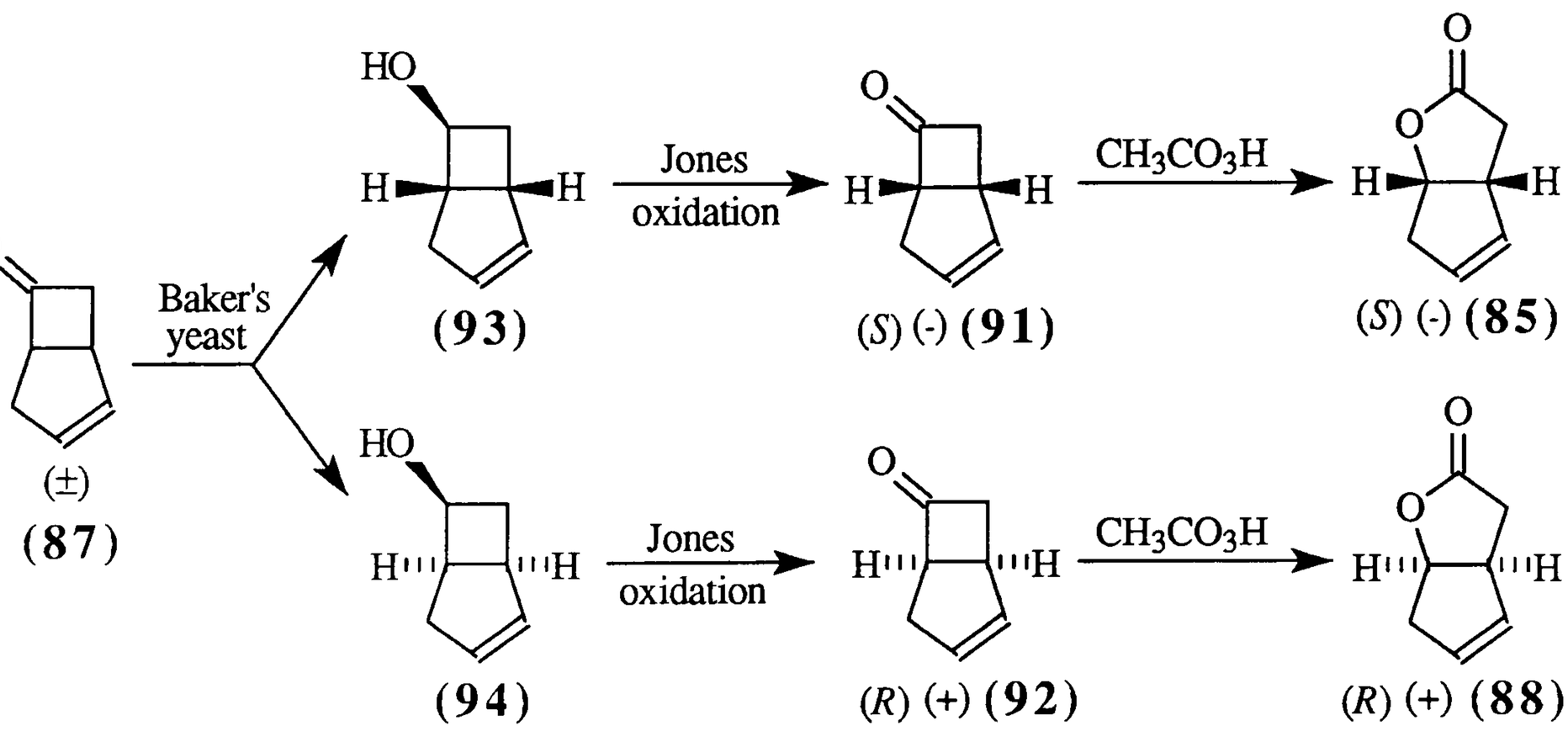
The starting material for the synthesis of lactone (**82**) was dichloroketone (**86**) prepared *via* a [2+2] cyclisation reaction between dichloroketene and cyclopentadiene. The work described in this thesis is with racemic material. The lactone denoted '(**82**)' is the racemate, and that labelled (**85**) the desired enantiomer. Even though all structures depicted are the desired enantiomers for the target molecules, in order to obtain the appropriate enantiomer a separation would have been required at some point, (unless homochiral starting material had been used). Both the (-) lactone (**85**) desired for the synthesis of the aglycone or mycinoic acids and the enantiomer (+) (**88**) are available commercially[54].

Several methods have been reported to obtain the homochiral bicyclic heptanone or lactone. *Endo*-α-hydroxysulfoximines **(89)** and **(90)** formed by Johnson and Zeller[55] from racemic ketone **(87)** were separated by column chromatography and the homochiral ketones **(91)** and **(92)** regenerated by thermolysis; however, the low yield of the products of sulfoximide anion attack prevents use of such a route on a large scale.



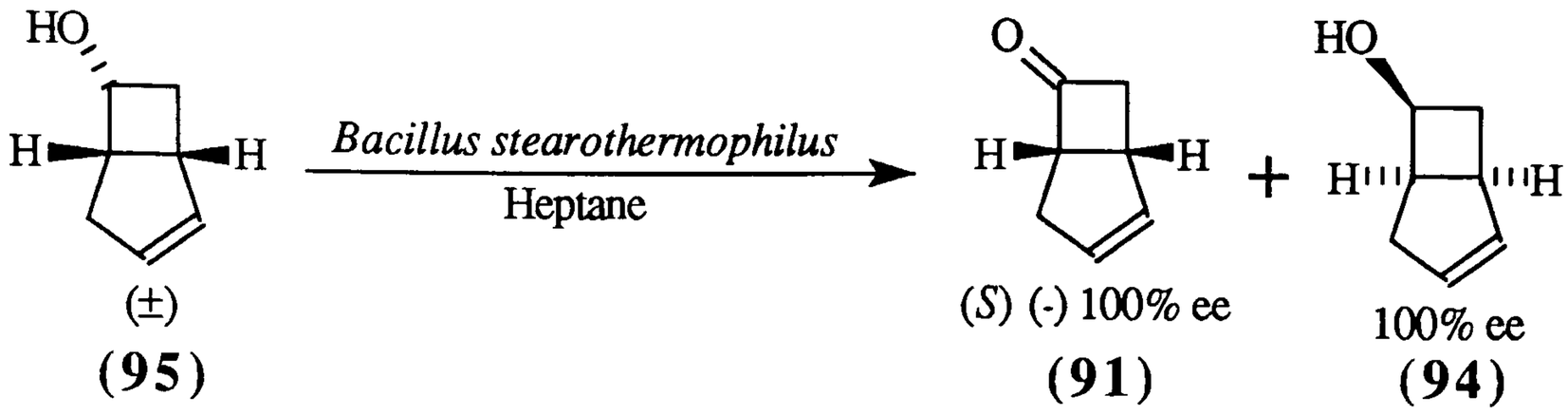
Scheme 1.11: Chemical resolution of the racemic ketone **(87)**.

A bakers yeast reduction of **(87)** followed by separation to the homochiral alcohols and reoxidation gave the enantiomeric ketones **(91)** and **(92)**, which were further oxidised to the lactones **(85)** and **(88)**[56].



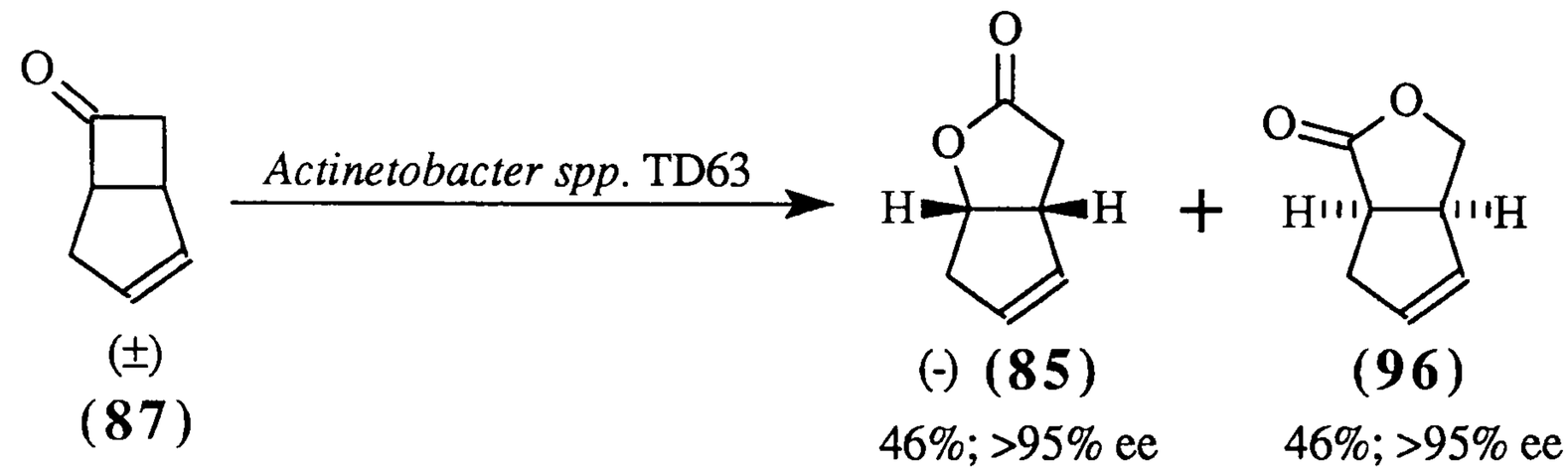
Scheme 1.12: Reduction of racemic ketone **(87)** and subsequent oxidations.

The racemate of the *endo*-alcohol (**95**) was selectively oxidised by a whole cell culture giving (**91**) and the alcohol (**94**); the latter might be chemically oxidised to the corresponding lactone (**88**) if so desired[57].



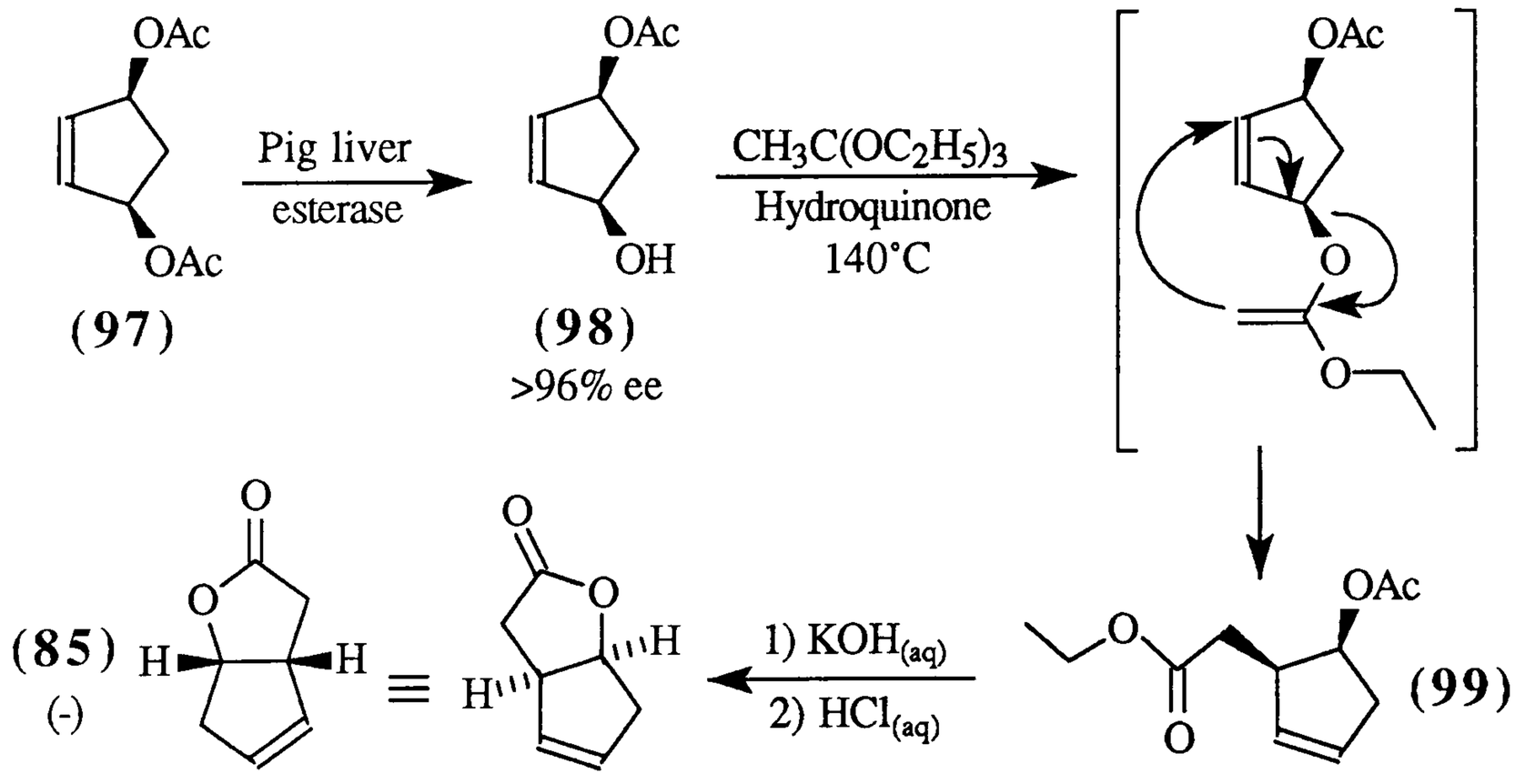
Scheme 1.13: Whole cell stereoselective oxidation of the racemic alcohol.

A whole cell culture can convert the racemic ketone to lactones[58]; however, whilst both yields and enantiomeric excesses were good, two regioisomers are obtained.



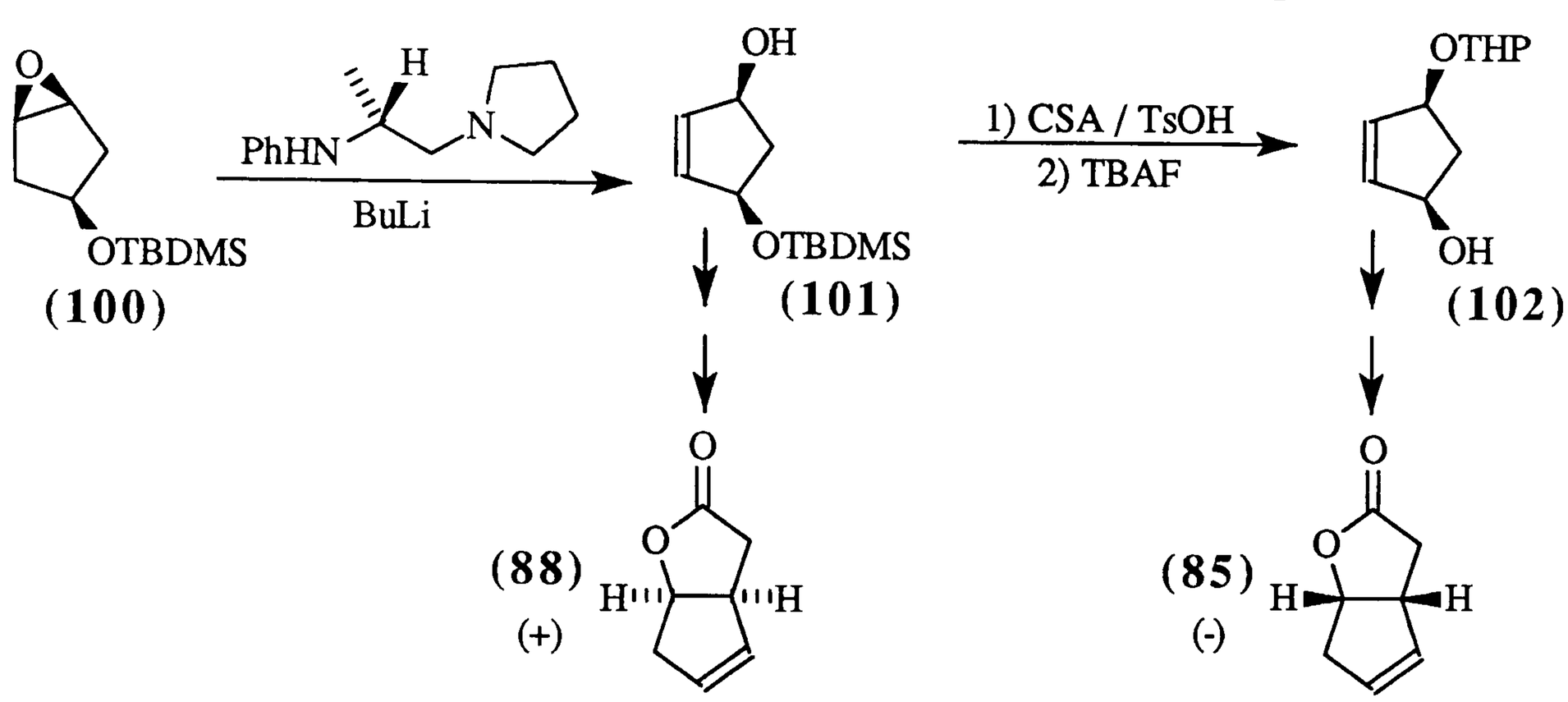
Scheme 1.14: Whole cell enzymatic Baeyer-Villiger of the racemic ketone (**87**)

The use of an esterase upon the achiral diester (**97**) gave the mono-ester (**98**)[59]; treatment of (**98**) as detailed in scheme 1.15 by Kondo[60] gave, *via* a Johnson ortho ester reaction and a Claisen rearrangement, (**99**) which led to homochiral (**85**).



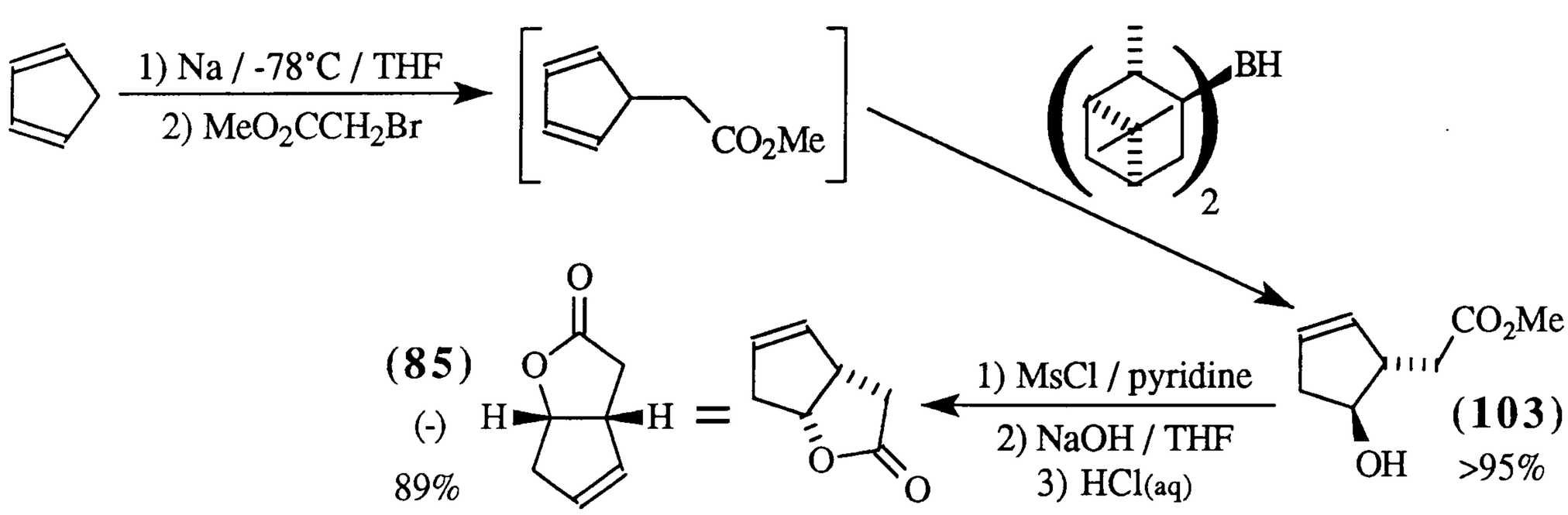
Scheme 1.15: Semi-synthetic route to enantiomerically pure lactone (**85**)[59,60].

A chemical synthesis of chiral lactones (scheme 1.16)[61] was based upon treatment of the prochiral epoxide (**100**) with a chiral base to form the chiral alcohol (**101**); this may be reacted with an ortho ester and then cyclized to the lactone (**88**), in a similar manner to scheme 1.15. The desired enantiomer (**85**) could be formed by basing the cyclization upon the alternate THP protected alcohol (**102**), developed as shown.



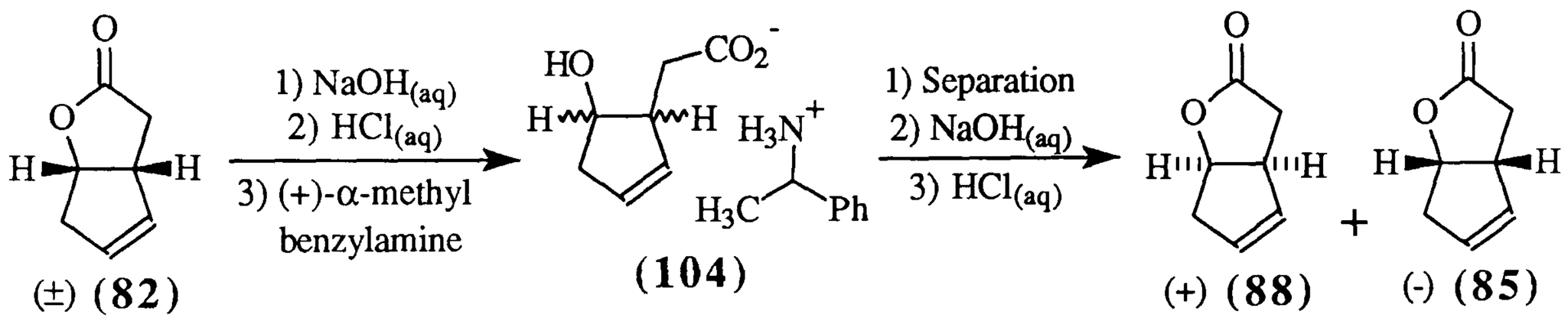
Scheme 1.16: Route to homochiral lactones from prochiral epoxide.

Use of the cyclopentadiene anion with methyl bromoacetate gave an ester which was reacted immediately with an asymmetric hydroboration agent to form the cyclopentenol derivative (**103**). Formation of the methanesulfonate ester and saponification of the carboxylate ester gave a carboxylate anion, which on treatment with mineral acid enabled esterification to form chiral lactone (**85**). The route enables the isolation of the cyclopentenol (**103**) as a stable intermediate for further purification if the hydroboration was not sufficiently enantioselective[62].



Scheme 1.17: Route to homochiral lactone (**85**) using asymmetric borane.

An alternative method was based on work by Corey[63]. Opening the racemic lactone **(82)** with hydroxide, acidification in the presence of ethyl acetate to give the hydroxy acid and reaction with (+)-α-methylbenzylamine formed the diastereomers **(104)**, which were separated by recrystallisation from ethyl acetate and methanol. The methylbenzylamine was removed by aqueous base and the enantiomeric lactones reformed by treatment with acid.



Scheme 1.18: Separation of homochiral lactones by formation of diastereomers.

PARTIAL SYNTHESIS OF MYCINOLIDE III AND THE MYCINOIC ACIDS

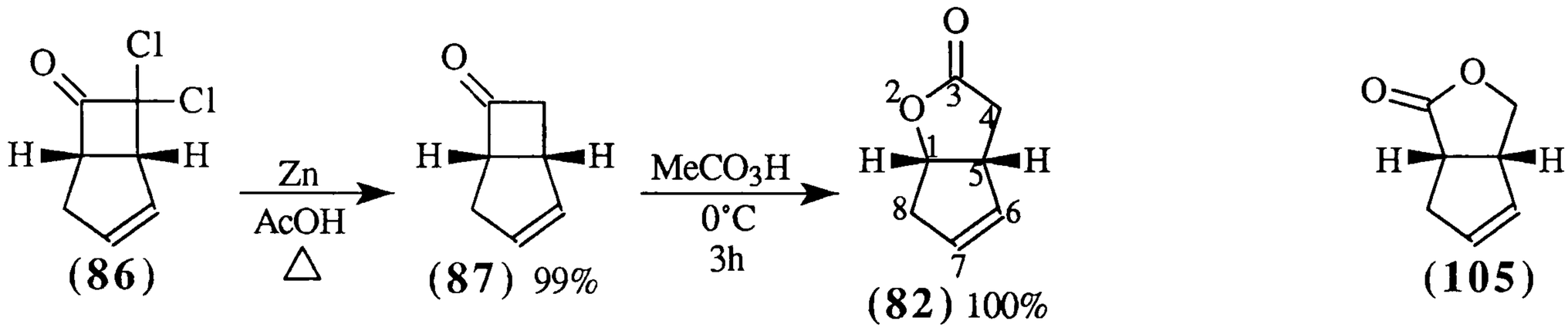
CHAPTER TWO:

Results and Discussion

2 Results and Discussion

2.1 Synthesis of Bicyclic Lactone (82)

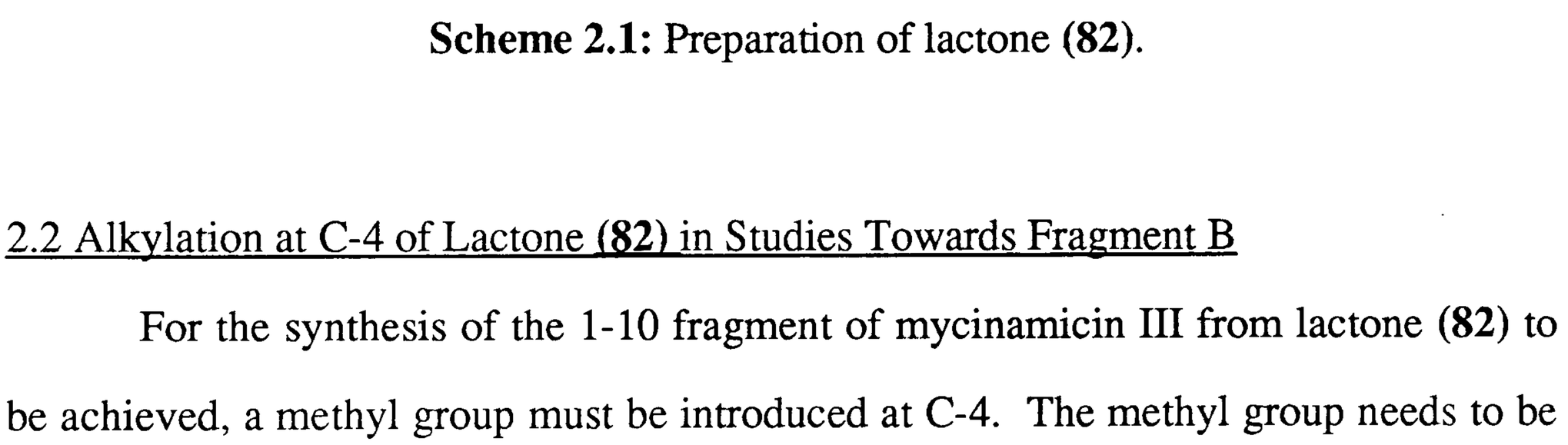
In order to obtain the lactone (**82**), the dichloroketone (**86**) was dechlorinated by treatment with zinc dust in acetic acid, and purification by distillation gave (**87**) in 99% yield (scheme 2.1). The distilled ketone was added to one equivalent of peracetic acid made *in situ* from hydrogen peroxide and acetic acid to give the lactone (**82**) in quantitative yield on work-up; no further purification was necessary. The complete regiocontrol of oxidation to form lactone (**82**) is due to the higher electron density at the bridgehead carbon, C(5), than at C(7) of ketone (**87**), thus C(5) migrates during the Baeyer-Villiger reaction. None of the regioisomer (**105**) was seen, unlike the *Acinetobacter spp*. oxidation of the racemate[64]. Nor was any epoxidation of the olefin seen if the reaction was held at 0°C.



Scheme 2.1: Preparation of lactone (**82**).

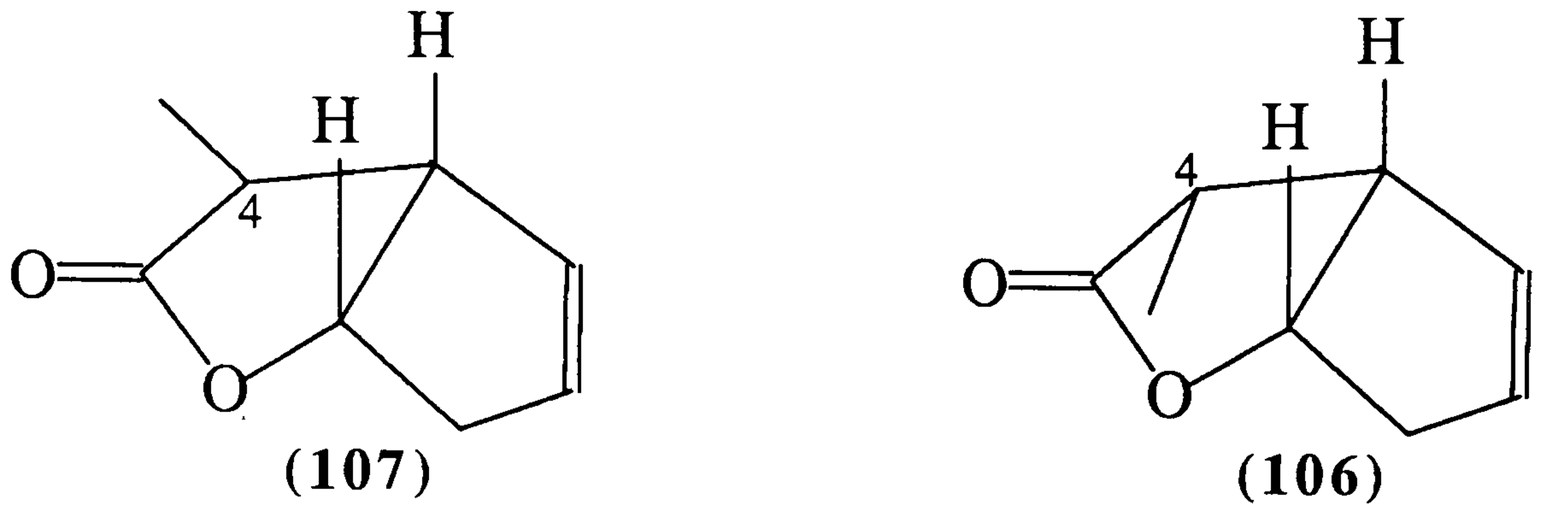
2.2 Alkylation at C-4 of Lactone (82) in Studies Towards Fragment B

For the synthesis of the 1-10 fragment of mycinamicin III from lactone (**82**) to be achieved, a methyl group must be introduced at C-4. The methyl group needs to be in the *endo* position (α) to give the 4(*S*)-methyl group in fragment B (**84**), as indicated in scheme 1.9.



Scheme 2.2: Proposed intermediates to Fragment B.

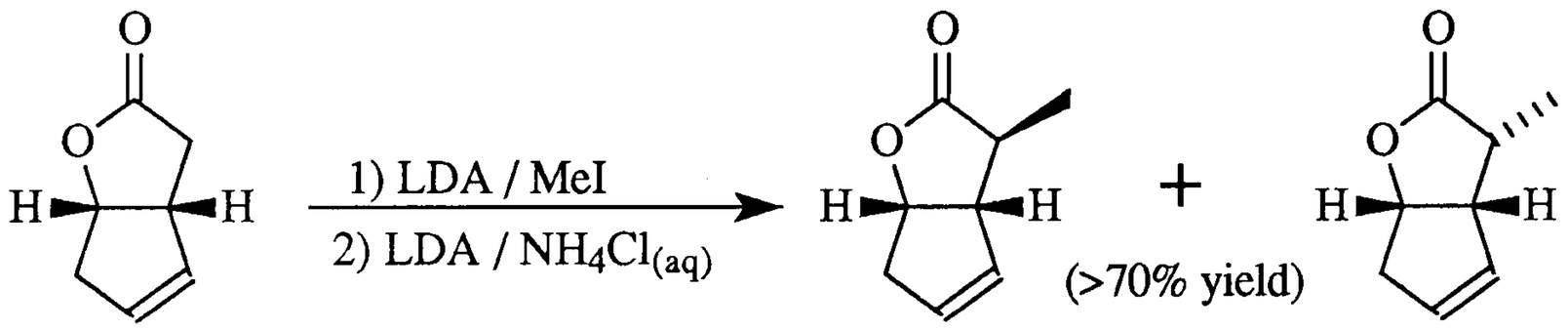
Thus to prepare (**106**) from lactone (**82**) it is necessary to introduce the methyl group to the more sterically hindered face of the molecule, not the more easily obtained *exo* derivative (**107**).



(107) **(106)**

Figure 2.1: Epimers of 4-methyl derivative of bicyclic lactone (**82**).

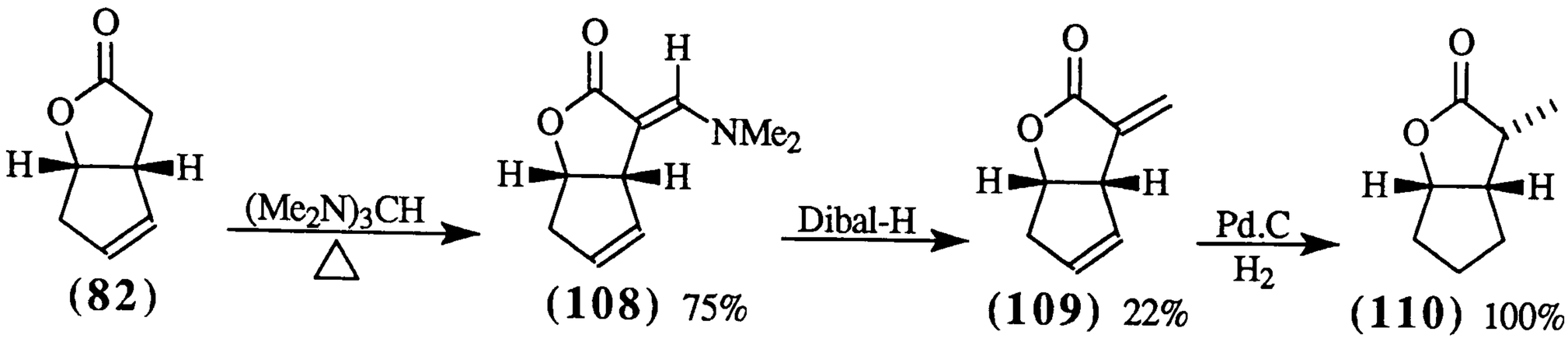
Previous studies[65] had shown that the 4β-methyl isomer (**107**) could be prepared directly in 100% yield by reacting lactone (**82**) with LDA and methyl iodide. Inversion of the 4β-methyl to the required α-stereochemistry was investigated via the intermediate enolate generated from lactone (**107**) with LDA. A range of electrophiles was examined to achieve protonation of the enolate from the β-face. However, the results were disappointing giving at best, a 78:22 mixture of the β:α-methyl derivatives ((**107**) and (**106**) respectively) when saturated ammonium chloride solution was used as the electrophile upon the enolate of (**107**).



(82) **(107)** 78 : 22 **(106)**

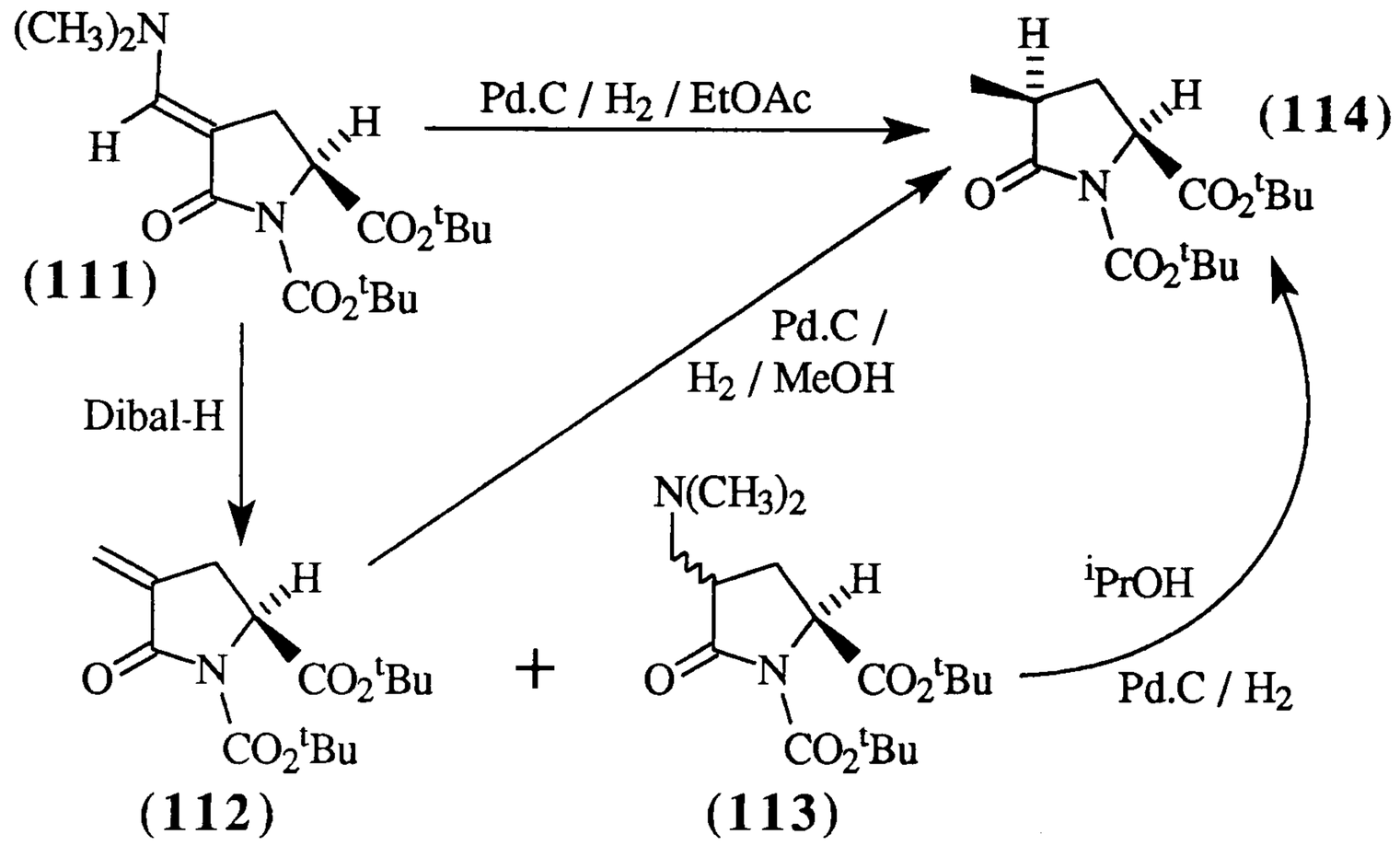
Scheme 2.3: Previous "one-pot" route to 4α-methyl lactone[65].

Since this method was unsatisfactory for the preparation of the 4α-methyl derivative, a different approach was investigated[65]. Enamine (**108**) was prepared from (**82**) in good yield using Bredereck's reagent[66]. However repeated attempts to convert (**108**) to (**109**) using Dibal-H, based upon the work of Ziegler and Fang[67], gave (**109**) in only 22% yield; catalytic hydrogenation of the alkene yielded (**110**) quantitatively.



Scheme 2.4: Previous synthesis of 4α-methyl lactone[65].

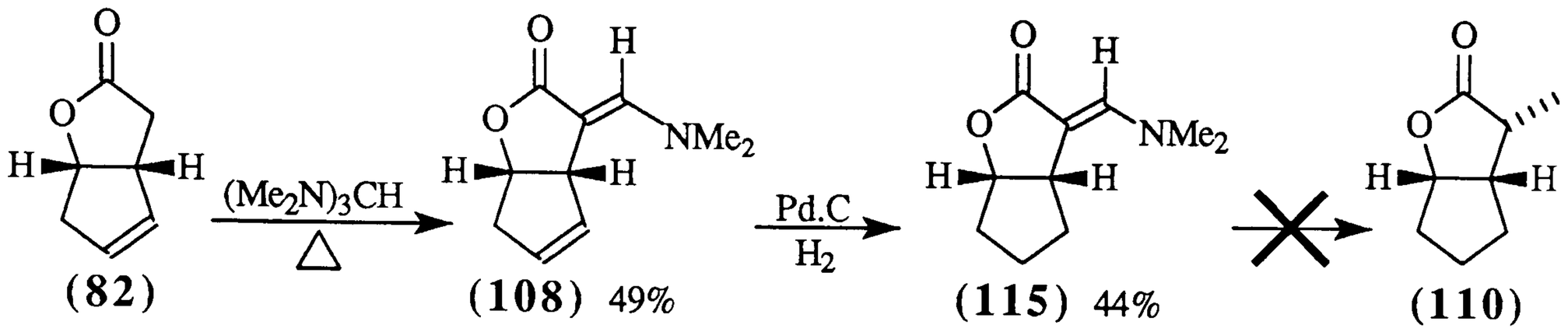
Hence if this approach to the 4α-methyl lactone is to be synthetically valuable, an improved method for the reduction of the enamine is required. Recently Young *et al.*[68] have reported that, although treatment of enamine **(111)** with Dibal-H did not give satisfactory yields of the olefin **(112)** (and by-products such as **(113)** were formed), direct reduction of **(111)** with 50%w/w palladium on carbon in ethyl acetate gave the required β-methyl derivative **(114)** in 78% yield.



Scheme 2.5: Young's reduction of enamino-*N*-carboxy-amide[68].

Hence our investigations into methods for alkylation at the *endo*-face of C-4 of lactone **(82)** began with the direct catalytic hydrogenation of enamine **(108)**. Treatment of **(82)** with Bredereck's reagent gave **(108)** in 49% yield. Stirring **(108)** in ethyl acetate with 10% palladium on calcium carbonate (20%w/w) under a hydrogen atmosphere led solely to reduction of the 6,7-double bond giving **(115)** in 44% yield. When palladium on carbon (50% w/w) was used as the catalyst in either ethyl acetate or a mixture of

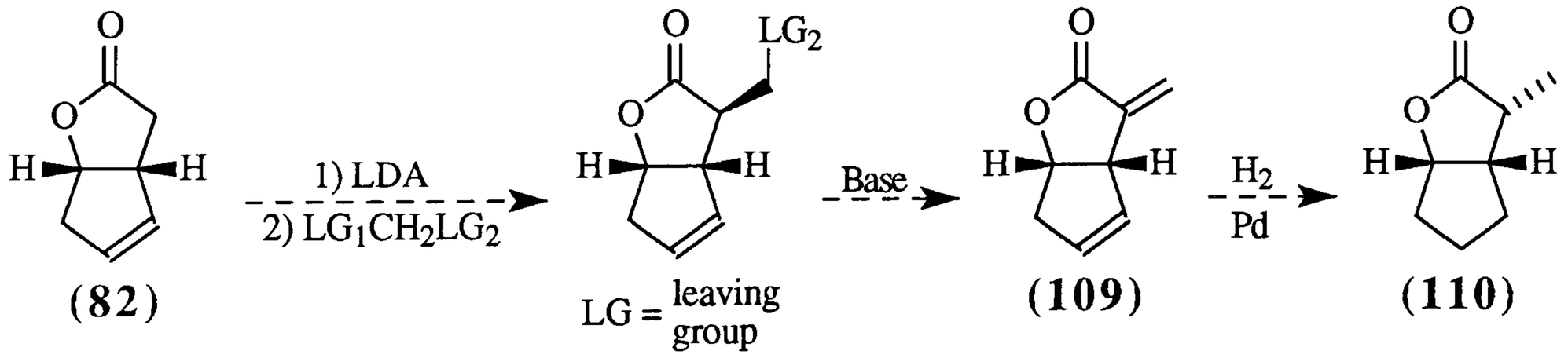
ethyl acetate and acetic acid, again enamine **(115)** was the only product recovered (in 53% and 92% yield respectively).



Scheme 2.6: Attempted reduction of vinylogous carbamate **(108)**.

Since no reduction of the carbamate had been observed when the reduction was carried out at atmospheric pressure, a medium pressure hydrogenation was attempted but again this led simply to reduction of the 6,7-double bond giving **(115)** in poor yield (33%); no other products could be identified by ^{1}H NMR spectroscopy. It is not clear why attempts to reduce enamine **(108)** under catalytic hydrogenation conditions failed when **(111)** was smoothly reduced under similar conditions[69]. However it was apparent that a different method for the introduction of a 4α-methyl substituent was required.

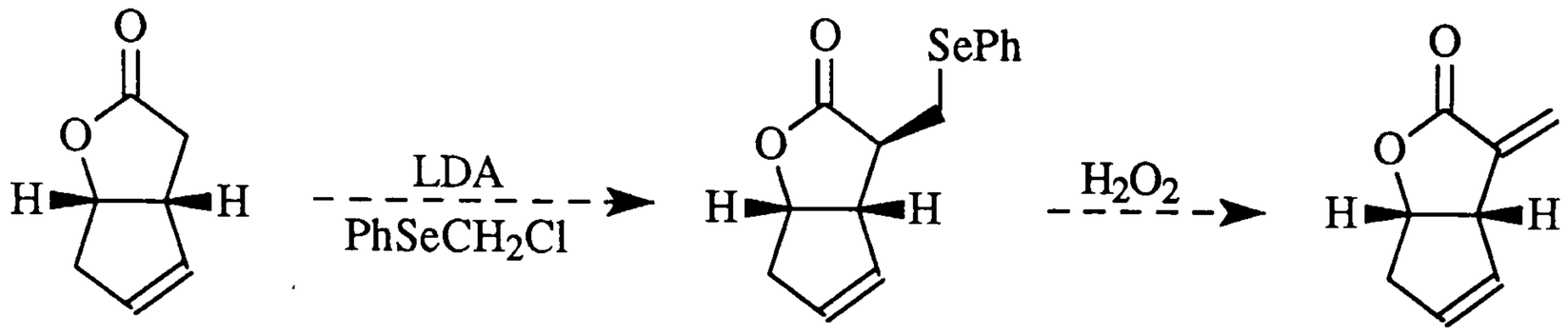
The second approach to the problem of introducing a 4α-methyl group involved alkylation of lactone **(82)** followed by elimination of a suitable leaving group to give the exocyclic methylene **(109)** (Scheme 2.7). From previous studies[65] it is known that catalytic hydrogenation of the double bond occurs from the *exo* face giving the 4α-methyl lactone **(110)**.



Scheme 2.7: Proposed route to 4α-methyl lactone.

Treatment of lactone **(82)** with LDA followed by either diiodomethane or chloroiodomethane gave a complex mixture of products which could not be identified by ^{1}H NMR spectroscopy.

Paterson and Fleming[70] have reported that α-methylenation of a γ-lactone may be achieved using chloromethylphenylsulfide as the electrophile and subsequent oxidation with sodium periodate to the sulfoxide and *syn* elimination to the α,β-unsaturated lactone. Grieco and Miyashita[71] have used a similar approach for α-methylenation using chloromethylphenylselenide.

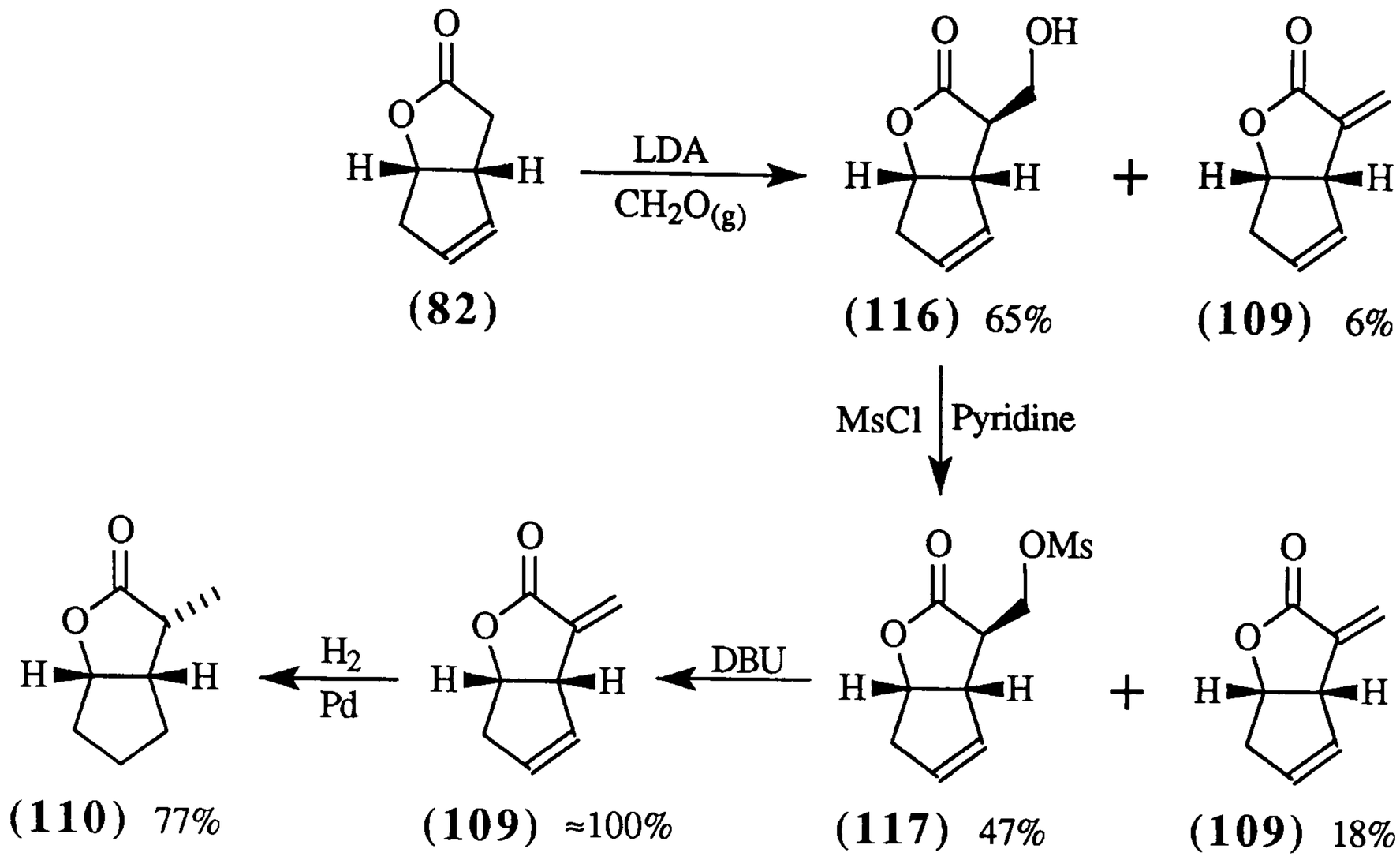


(82) **(109)**

Scheme 2.8: Proposed route using chloromethylphenylselenide.

Chloromethylphenylselenide was prepared according to the procedure described by Beckwith and Pigou[72]. Reaction of diphenyldiselenide with sodium borohydride in methanol gave the phenylselenide anion, which was added to DCM to form chloromethylphenylselenide. Treatment of lactone **(82)** with LDA followed by chloromethylphenylselenide simply returned starting material. To determine if the problem was the lack of activity of chloride as a leaving group, a primary alkyl chloride, 2-phenylethyl chloride, was reacted with the γ-enolate; again only starting material was recovered. This indicates that an electrophile with a better leaving group than chloride was required to react with the enolate.

It has been reported[73] that α-methylenation of a δ-lactone may be achieved by generation of the enolate with LDA and quenching with gaseous formaldehyde followed by dehydration of the resultant β-hydroxy-lactone. Treatment of lactone **(82)** with LDA and gaseous formaldehyde (generated from paraformaldehyde) gave a mixture of two products which were separated by flash chromatography. The ^{1}H NMR spectrum of the more polar product showed a characteristic ABX pattern (3.88ppm, dd, *J* 11 and 4.5, 4-C<u>H</u>HOH and 4.02ppm, dd, *J* 11 and 4.5, 4-CH<u>H</u>OH) and was assigned as the β-hydroxy lactone **(116)**. The less polar product was the unsaturated lactone **(109)** (5.62ppm, d, *J* 2, 4-C<u>H</u>H and 6.12ppm, d, *J* 2, 4-CH<u>H</u>).



Scheme 2.9: Pathway to 4α-methyl lactone **(110)**.

Alcohol **(116)** was treated with methanesulfonyl chloride in pyridine to give mesylate **(117)** and the elimination product **(109)** which were separated by flash chromatography. Reaction of the mesylate with DBU gave the alkene **(109)** in quantitative yield. Catalytic hydrogenation of **(109)** gave 4α-methyl lactone **(110)** in 77% yield. Even though this has reduced the 6,7-double bond, this was not important in the final synthesis of the target 4,6,8-trimethylated lactone as the double bond would be utilised by other reactions prior to the introduction of the 4α-methyl group.

The alkene **(109)** is potentially a valuable intermediate in the synthesis of a range of macrolides. Since the exocyclic methylene is conjugated, it will be susceptible to copper catalysed conjugate additions and so could be used to allow the introduction of other groups for microbiological screening.

Although the introduction of the 4α-methyl substituent has been achieved, the overall yield from the lactone **(82)** was rather disappointing (42% overall yield). A more direct approach would involve introduction of the 4β-methyl substituent via enolate chemistry (i.e. correct regiochemistry but incorrect stereochemistry), and inversion of the stereochemistry by reforming an enolate and protonation from the less hindered *exo*-face at C-4. As stated at the beginning of this section this had been

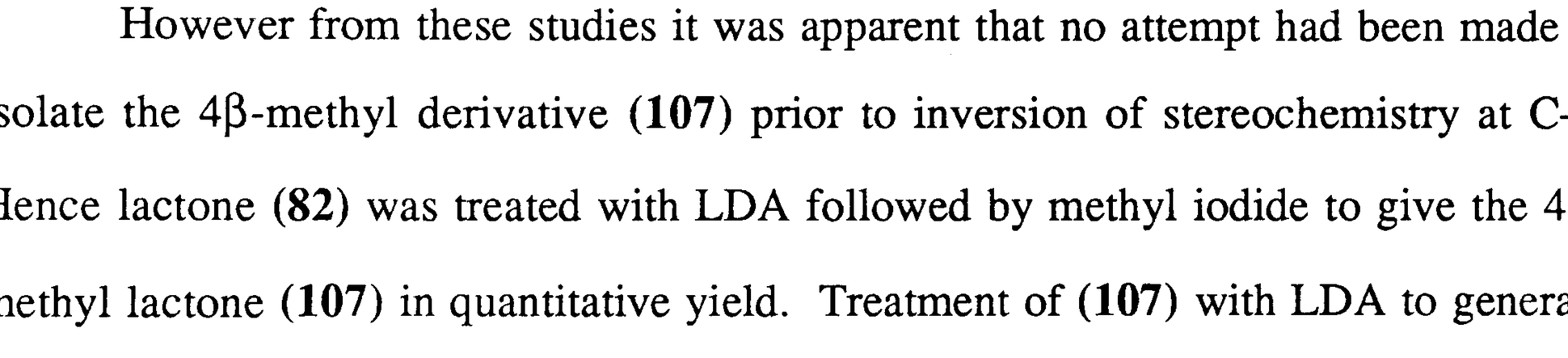
previously attempted with only limited success by Fraser[65]. The electrophile added was saturated ammonium chloride; use of other proton sources (water or dilute hydrochloric acid) gave lower yields of products.

Reaction time before electrophile added (minutes)	Temperature when quenched (°C)	% 4α-methyl (**106**) isomer
2	-78	0
10	-25	9
30	-5	22
60	+2	19

Table 2.1: Treatment of lactone (**82**) with LDA and methyl iodide, then LDA and saturated ammonium chloride solution by Fraser[65].

From the data obtained it appears that using longer reaction times and a higher temperature allows more of the *endo* isomer to be formed.

However from these studies it was apparent that no attempt had been made to isolate the 4β-methyl derivative (**107**) prior to inversion of stereochemistry at C-4. Hence lactone (**82**) was treated with LDA followed by methyl iodide to give the 4β-methyl lactone (**107**) in quantitative yield. Treatment of (**107**) with LDA to generate the enolate and quenching with water indeed led to selective protonation of the *exo*-face at C-4 giving a 98:2 mixture of the α:β-isomers, (**106**) and (**107**) respectively (scheme 2.10). The products were separable by flash chromatography. The percentage of each isomer was calculated from the integrals of the methyl doublets of the 4α- and 4β-methyl isomers in the ^{1}H NMR spectra (1.27ppm and 1.38ppm respectively).



(**82**) (**107**) 100% (**106**) 98 : 2 (**107**)

Scheme 2.10: Route using LDA / methyl iodide to unsaturated lactone (**106**).

It was fascinating to compare the results from the "one-pot reaction" (in which lactone **(82)** was treated with LDA / MeI then LDA / $NH_4Cl_{(aq)}$ giving a 22:78 mixture of **(106)** : **(107)**, scheme 2.3)[65] with the two step process (in which the 4β-methyl derivative **(107)** was isolated prior to reaction with LDA/H_2O). The difference may be due to the presence of either excess diisopropylamine or lithium iodide in the "one-pot" procedure which are not present in the inversion of stereochemistry step of the "two-stage" reaction. This was confirmed by treatment of **(107)** with LDA in the presence of 1 equivalent of diisopropylamine giving, after a standard work-up, a 49:51 mixture of **(106)** : **(107)**. When the enolate of **(107)** was generated under standard conditions but diisopropylamine was then added at -78°C rather than water, then a yield of 60% of **(106)** was obtained. Thirdly, diisopropylamine was used to quench the enolate and the reaction then heated to 67°C, which gave 43:57 ratio **(106)** : **(107)**. There were no significant differences between the ratio of products, though the use of higher temperature perhaps should have increased the proportion of **(107)**. However, all reactions showed a lower degree of conversion from **(107)** to **(106)** than using water as the electrophile, due to diisopropylamine being a weaker acid and more sterically hindered than water. Similarly, only 55% conversion of **(107)** to **(106)** was obtained using *t*-butyl alcohol as the electrophile.

In contrast, when the reaction was repeated in the presence of an excess of lithium iodide and quenching with water, >85% of the desired 4α-methyl derivative **(106)** was obtained. Hence the presence of excess diisopropylamine leads to a different ratio of products in the "one-pot" versus the "two-pot" reaction.

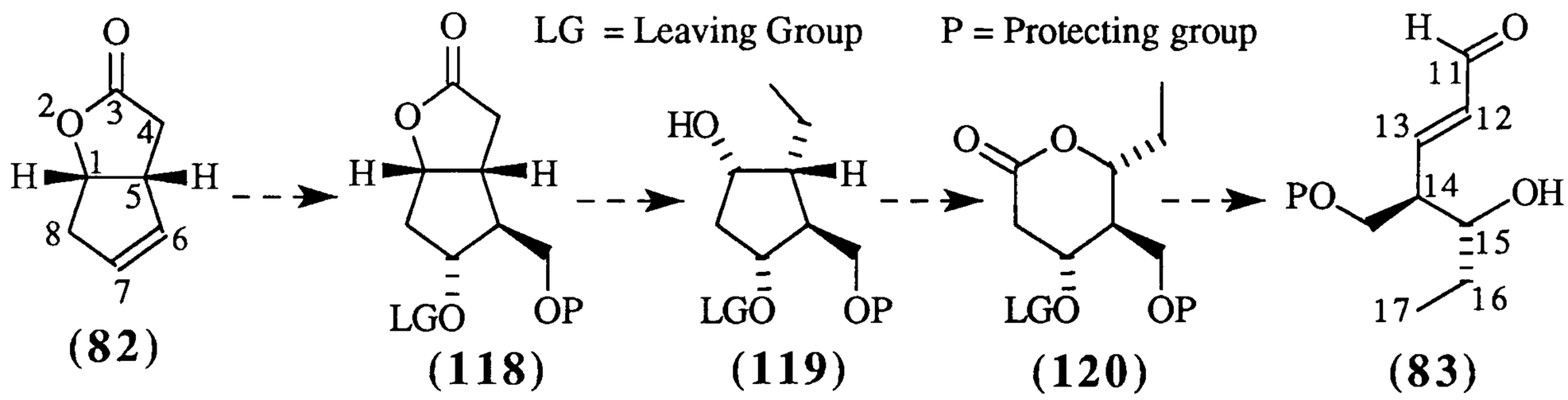
Thus the preferred route for the introduction of a methyl group into the 4α position of lactone **(82)** is to prepare the 4β-methyl isomer **(107)** with LDA/MeI and then to invert the methyl via formation of the enolate giving the required 4α-methyl isomer **(106)** in 97% overall yield from **(82)** (scheme 2.10).

The introduction of further substituents and work towards the synthesis of fragment B (**84**) of mycinolide III is reported in the PhD theses of C. Clissold[74] and J. Fraser[65].

2.3 Studies Towards the Synthesis of Fragment A (C_{11} to C_{17})

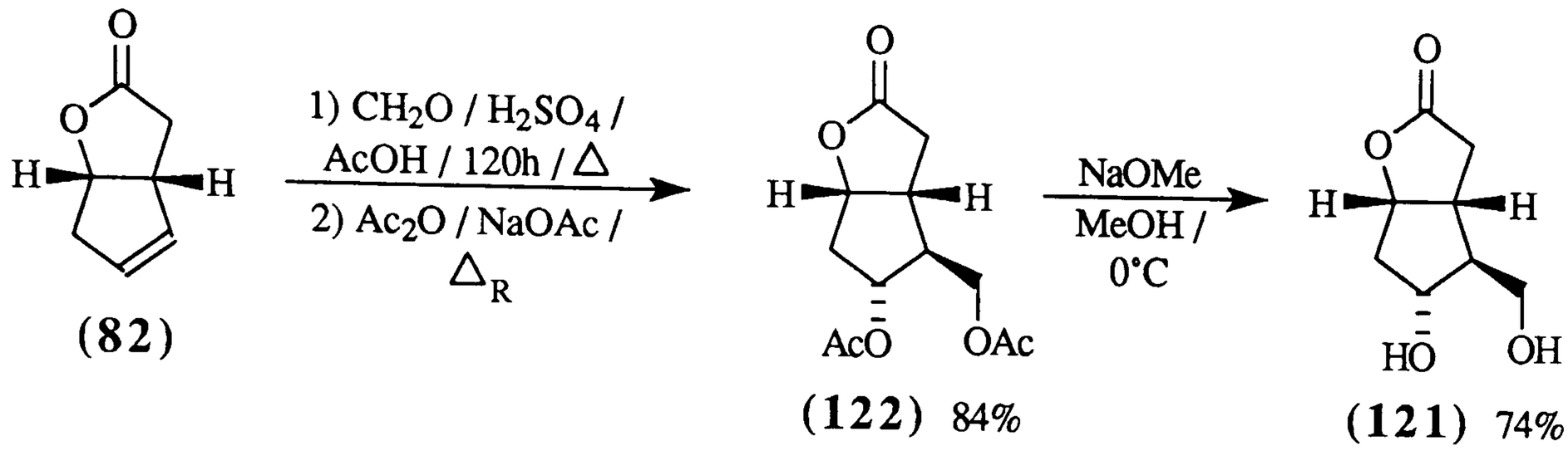
2.3.1 Synthesis of Benzylidene Protected Diol (**127**)

The proposed route for the synthesis of fragment A (C_{11}-C_{17}) (**83**) of mycinolide III (**39**) was based upon previous work by Robinson in our group[75] (scheme 2.11). The strategy involved introduction of a hydroxymethyl group at C(6) (to become the 14-hydroxymethyl in mycinolide III (**39**)) and a potential leaving group at C(7) (to aid formation of the olefin at C(12) to C(13) in (**83**)). Manipulation of lactone (**118**) to a cyclopentanol (**119**) would then be followed by sequential oxidations to a ketone and a regioselective Baeyer-Villeger reaction to δ-lactone (**120**). Finally, reduction of the lactone to a lactol and elimination would give the α,β-unsaturated aldehyde (**83**) required for macrocylisation to form (**39**) (scheme 1.9).



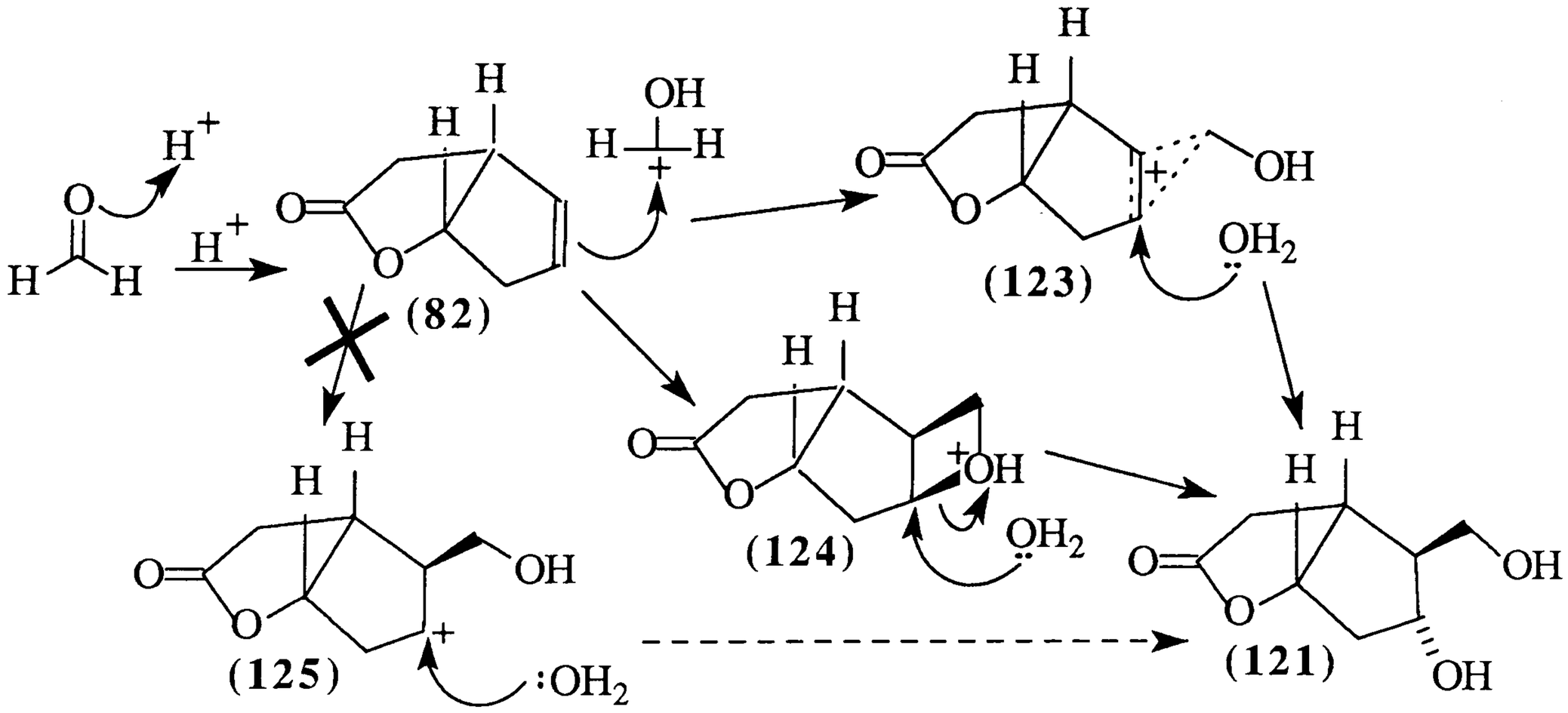
Scheme 2.11: Proposed strategy for the synthesis of fragment A (**83**).

The lactone (**82**) was reacted with formaldehyde and sulfuric acid in a Prins[76] reaction to give the diol (**121**) which was acetylated *in situ* to form the diacetate (**122**), according to a method of Kovacs[77] (scheme 2.12). The diol (**121**) is so polar that it is difficult to extract directly from the reaction mixture, so the product had to be acetylated *in situ* to enable extraction as the diacetate (**122**). The diacetate was then treated with sodium methoxide to give (**121**).



Scheme 2.12: Route to diol (**121**).

Although this reaction sequence to form (**121**) from the lactone (**82**) was reliable, the overall yield of diol (**121**) was only 62%. To investigate whether this yield may be improved by isolation of the diol (**121**) directly from the Prins reaction, on one occasion only sodium acetate, but not acetic anhydride, was added during work-up, giving diol (**121**) in only 53% yield after column chromatography. It has been proposed that the Prins reaction upon lactone (**82**) proceeds via either the cyclopropylinium (**123**)[78] or the oxetanium (**124**)[79] cations, but mechanistic studies have shown a simple secondary carbocation (**125**) is not the intermediate (scheme 2.13). This may account for the lack of observation of regio and stereoisomers of (**122**).

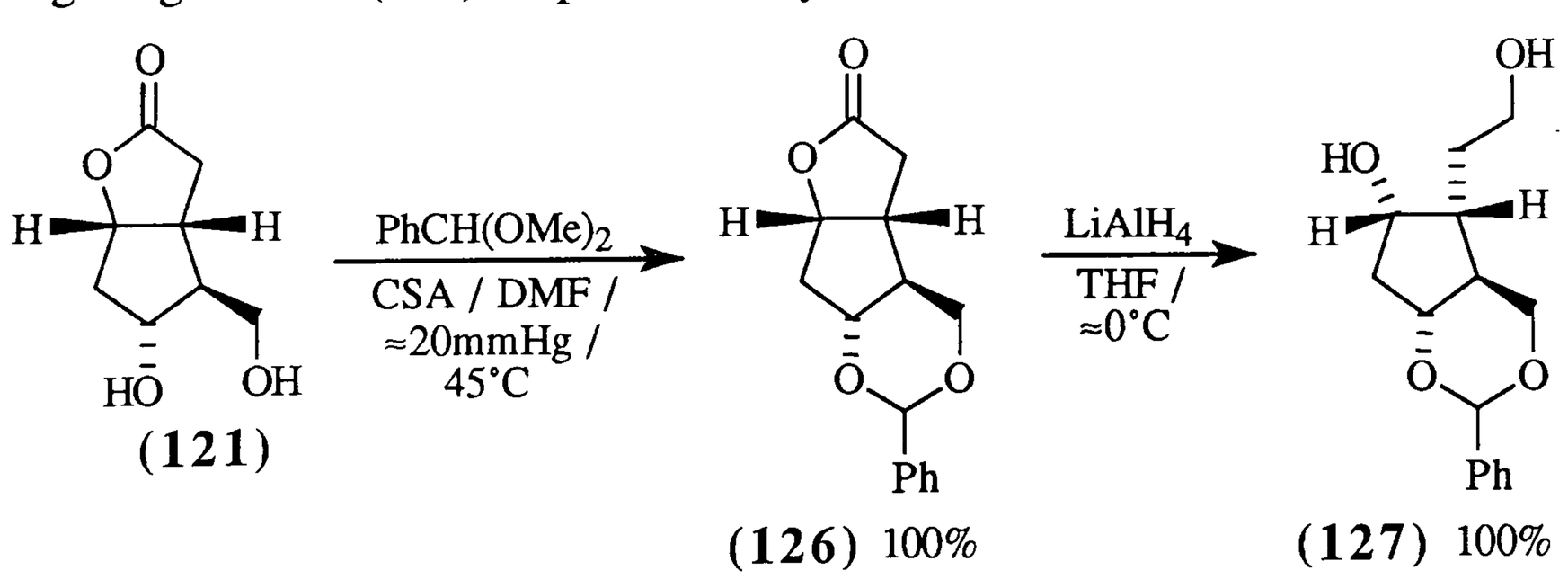


Scheme 2.13: Proposed mechanisms for formation of the diol (**121**) from lactone (**82**).

The next step in the proposed synthesis of mycinoic acids and fragment A of mycinamicin III was to protect the hydroxyl groups of the diol (**121**). Previous investigations by Robinson[75] had shown that when the primary alcohol of (**121**) was

protected as the TBDMS ether, the reaction with 2-methoxyethoxymethyl chloride and diisopropylethylamine caused migration of the silicon group to the secondary alcohol. Thus a new stable protection group was required. Treatment of diol (**121**) by Robinson with dimethoxypropane and *para*-toluenesulfonic acid did not give the desired ketal, however, the addition of a benzylidene group to give (**126**) was achieved using α,α-dimethoxybenzaldehyde acetal and CSA; use of zinc chloride with benzaldehyde (with or without azeotropic distillation), or benzal bromide and pyridine were far less successful[75].

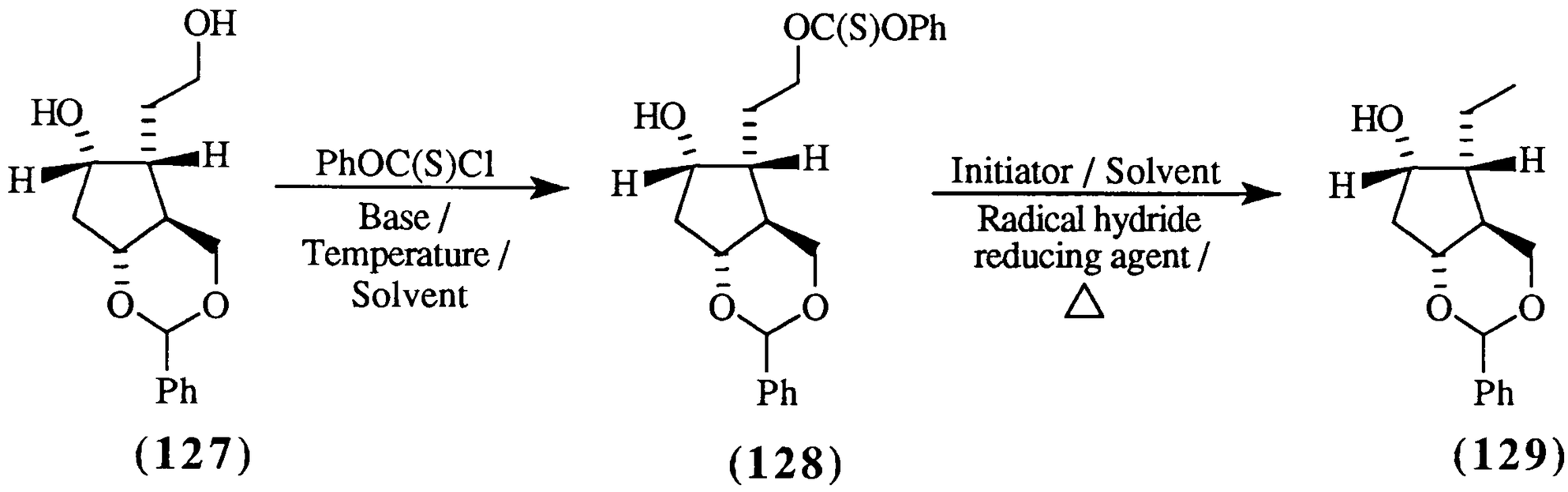
In this project, it was found that treatment of (**121**) with α,α-dimethoxybenzaldehyde acetal and CSA in DMF at 45°C under a vacuum of approximately 20mmHg gave the acetal (**126**) in quantitative yield on successive occasions (scheme 2.14). However, although this reaction had been repeated twenty-four times, in the latter stages of this research the acetal inexplicably failed to form. The reagents and starting materials were pure by ^{1}H NMR spectroscopy and melting point determination. Hence an alternative appoach to (**126**) was examined using PhCHO in the presence of CSA which gave the required product, albeit in only 50% yield. A reaction analogous to that with benzaldehyde was attempted using 2-nitrobenzaldehyde to form a derivative, in which the aromatic protecting group can be removed by *uv* irradiation[80]. However, none of the nitroaromatic acetal product was isolated, and only 22% of the starting material (**121**) was recovered. The next stage in the proposed pathway invoked formation of a ethanolic group for reduction to the ethyl group. The benzylidene protected derivative (**126**) was reduced with lithium aluminium hydride giving the diol (**127**) in quantitative yield (scheme 2.14).



Scheme 2.14: Synthesis of diol (**127**).

2.3.2 Preparation of Cyclopentanol (129) from Diol (127)

The next stage of the synthesis required the selective deoxygenation of the primary alcohol of **(127)** (which will be the 15 position in mycinamicin III) in the presence of the secondary alcohol. The deoxygenation procedure used previously[75] was to prepare the thiocarbonate **(128)** from the diol **(127)** using phenyl thionochloroformate and pyridine in DCM at 0°C followed by reduction under radical conditions with tri-*n*-butylstannane (scheme 2.15). On repeating the first stage of this reaction, the thiocarbonate **(128)** was isolated in only 50-55% yield (compared with 74% yield reported by Robinson[75]). Hence a range of different conditions were tried in order to improve the yield.



Scheme 2.15: Overview of route to cyclopentanol **(129)**.

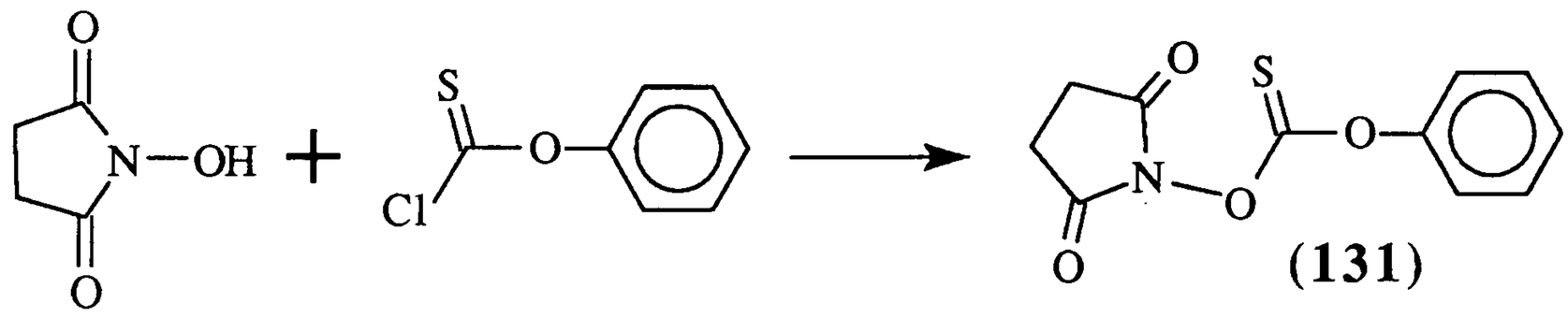
Due to the diol **(127)** being poorly soluble in DCM (indeed without the presence of pyridine it would not dissolve at all), a variety of more polar solvents were used in attempts to improve yield of the thiocarbonate **(128)**, see table 2.2. Either the use of acetonitrile or the absence of solvent gave even lower yields of product than other conditions, but no consistent improvement was shown by any one solvent system. In an attempt to improve the yield, the reaction was repeated at a lower temperature than 0°C, which should further have favoured the esterification of the primary alcohol and not the secondary alcohol. The alcohol **(127)** was completely insoluble at -78°C in DCM (even with the addition of base), so a more polar solvent was used, either a mixture of DCM & THF (1:1), acetonitrile or THF alone. However, the lowest temperature that enabled **(128)** to form was -12°C, and yields at this temperature were no improvement upon reaction at 0°C, except under use of ether as solvent. Reaction at room temperature

enabled formation of the *bis* product **(130)** also, although the maximum yield obtained was only 8%.

Solvents used	Two highest yields obtained
Acetonitrile	41 & 19%
Chloroform	86 & 43%
DCM	55 & 52%
DCM & ether	40%
DCM & THF	71&57%
Ether	90&79%
THF	70&68%
Toluene	67%
None	39&6%

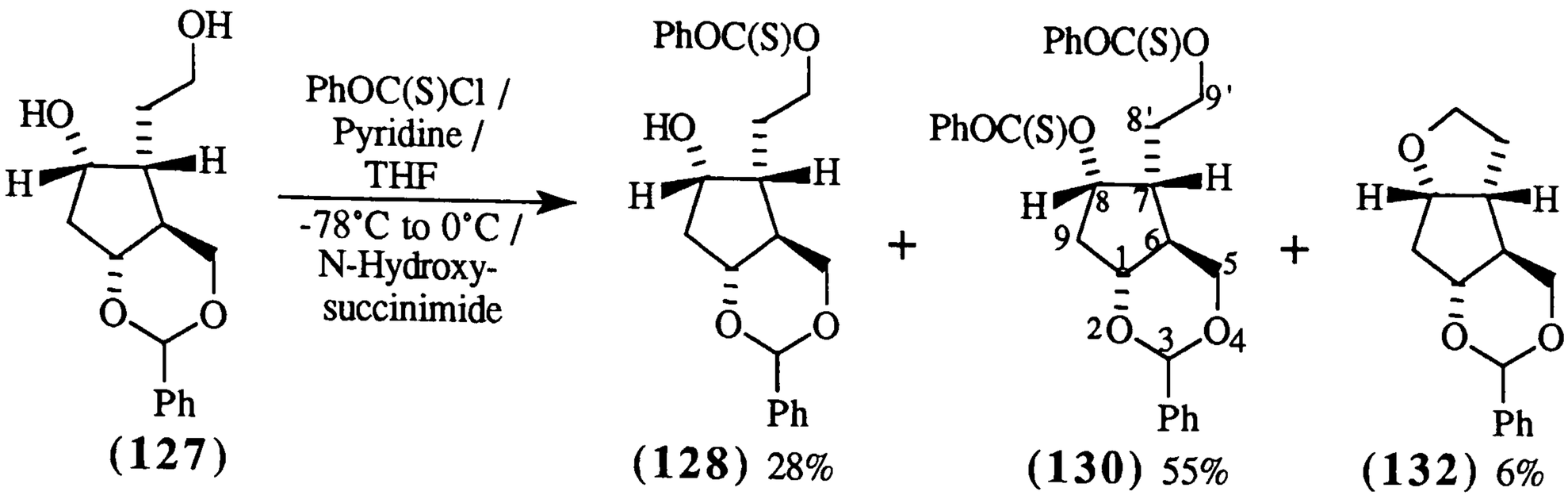
Table 2.2: Effect of solvent variation on yield of phenoxythiocarbonate **(128)**.

As the anion of N-hydroxysuccinimide is a better leaving group than a chloride ion[81], formation of the more reactive intermediate **(131)** (scheme 2.16) was examined.



Scheme 2.16: Formation of intermediate **(131)**.

However, upon treatment of diol **(127)** with phenyl chlorothionoformate, pyridine and N-hydroxysuccinimide, the yield of the monothiocarbonate **(128)** fell to 28% and the yield of the di-protected derivative **(130)** increased to 55% (scheme 2.17), as well as the cyclic ether **(132)** also being formed. *Bis*thiocarbonate **(130)** was readily identified in the ^{1}H NMR spectrum by the downfield shift of the signal assigned to 8-H from 4.26ppm in the monothiocarbonate **(128)** to 5.63ppm in **(130)**. The intermediate **(131)** appeared too effective an electrophile and enabled reaction of both hydroxyl groups, hence the use of N-hydroxysuccinimide was not repeated.



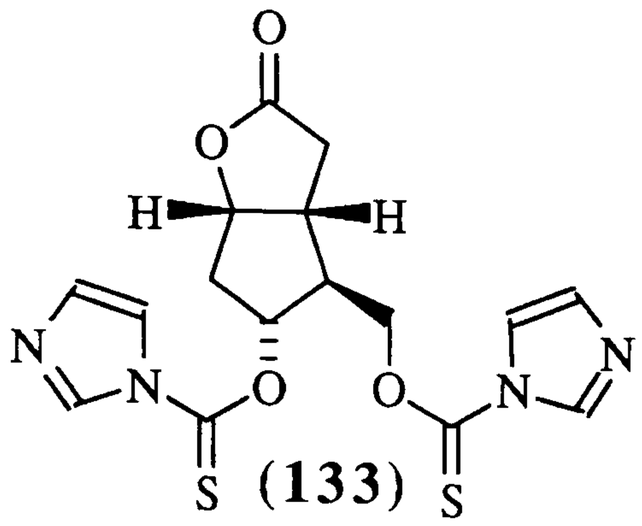
Scheme 2.17: Use of N-hydroxysuccinimide on thiocarbonate formation **(128)**.

The use of one equivalent of a metal hydride (as an alternative base to pyridine) with 18-c-6 and phenyl chlorothionoformate upon **(127)** was undertaken, as an alkoxide anion would be a more effective nucleophile than the free alcohol, and kinetic control should ensure preferential attack by the far less hindered primary alkoxide. However, results were far worse than with organic base: sodium hydride gave only 30% of the required product **(128)** and 27% starting material was returned, whilst potassium hydride yielded only 17% of **(128)**, with no other products found.

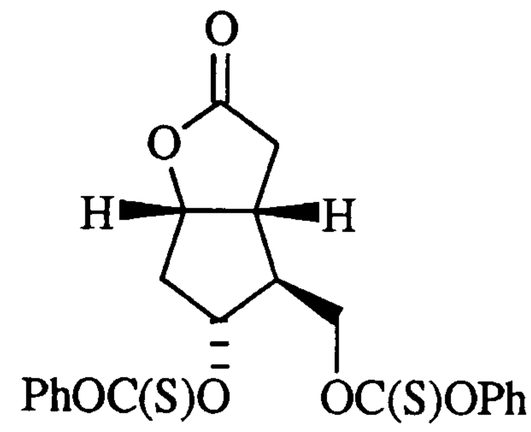
In an attempt to check the displaced chloride anion was not affecting the thiocarbonate formation, the diol **(127)** was reacted with silver fluoride, pyridine and phenyl chlorothionoformate, a technique based upon work by Suzuki *et al*[82]; however, phenoxythiocarbonate **(128)** was obtained in 22% yield only, with no other compounds identified from a complex mixture.

Use of DMAP (and pyridine as co-base) in the reaction with phenyl chlorothionoformate and diol **(127)** did not increase yields of **(128)** appreciably (40% and 44%), whilst replacing the aromatic bases with diisopropylamine gave a 51% yield of thiocarbonate **(128)** (plus 16% starting material was returned); the results suggest formation of the *N*-thiocarbonyl quarternary ammonium derivative of the base was not a factor in the yields of **(128)** being below quantitative. However, the use of imidazole was more beneficial, the thrice performed syntheses with this as base had yields of thiocarbonate of 90, 86 and 68%. This may not be due to the higher pK_a of the conjugate acid of imidazole (6.95) compared to pyridine (5.25)[83], as the more basic diisopropylamine gave a lower yield of **(128)** than pyridine, but the improved yield

using imidazole was perhaps due to the low steric bulk of the five membered ring. The preparation of a dithioimidazolide was not attempted, as Fraser[65] had shown addition of 1,1'-thiocarbonyldiimidazole to the diol **(121)** to give **(133)** had occurred in lower yield than the corresponding yield of diphenylthiocarbonate **(134)**; also the radical reagents could not reduce either of the *bis*-thiocarbonyl compounds to alkyl derivatives.



(133)



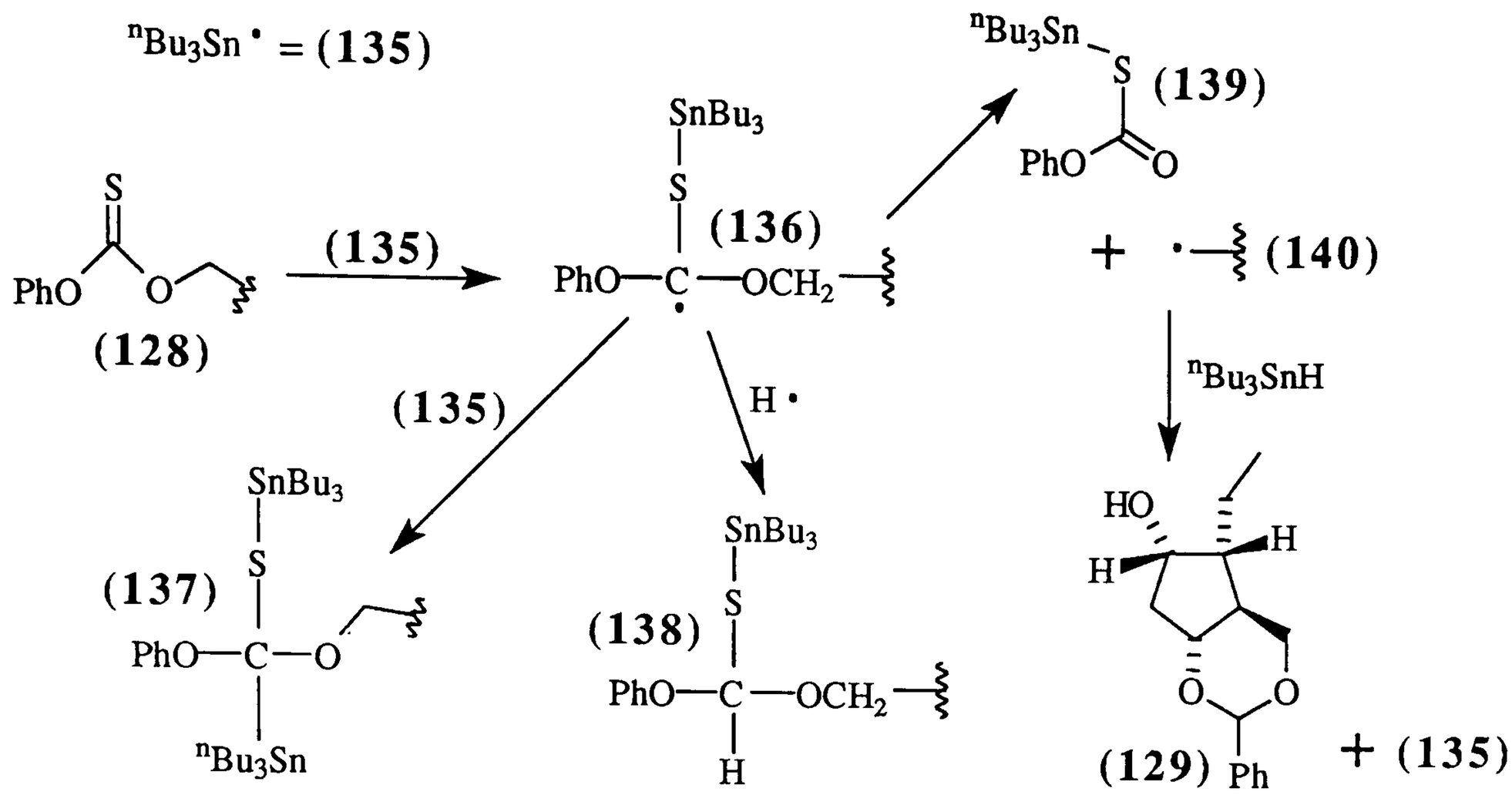
(134)

Figure 2.2: *Bis*-thiocarbonyl products prepared previously by Fraser[65].

Hence the use of the phenyl chlorothionoformate, imidazole and incongruously ether, a solvent in which the starting diol **(127)** would not dissolve without addition of other reagents, stirred under a nitrogen atmosphere at -12 to 0°C for several hours was the most efficient method to obtain thiocarbonate **(128)** giving the required product in 90% yield.

Having optimised the selective formation of the thiocarbonate on the primary alcohol, disappointingly it was found that free-radical reduction of **(128)** with tri-*n*-butyl tin hydride, the Barton-McCombie reaction, gave variable results ranging from 0-75% of the desired product **(129)**, as noted previously[84]. Thus numerous changes were made to the technique in an effort to improve both yield and the reproducibility of the reaction (scheme 2.15).

Unreacted starting material **(128)** was but rarely returned, however the reaction frequently produced complex degradation products, some of the identified and postulated products and intermediates[85] are depicted in scheme 2.18.

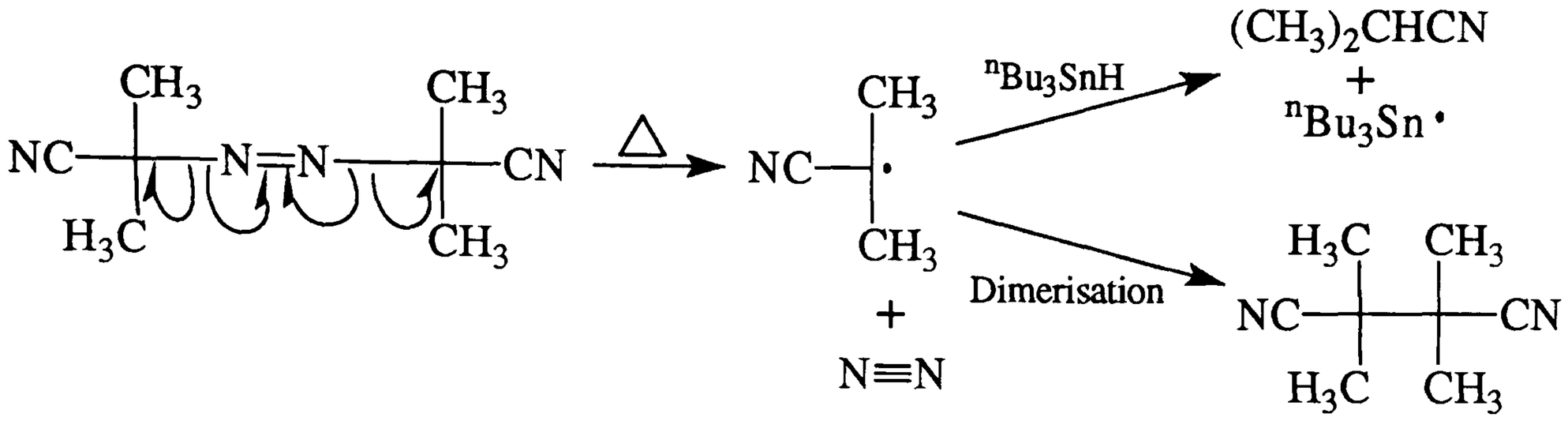


Scheme 2.18: Products, postulated and obtained, from radical reduction of **(128)**.

If insufficient free radical initiator, AIBN, was being cleaved to generate the radical, which can enable tri-*n*-butyltin hydride to form tributylstannic radical **(135)**, the thioformate **(128)** would not be able to react (schemes 2.18 and 2.19). To determine if sufficient AIBN was breaking down, AIBN was heated to reflux in benzene for 2 hours; analysis by NMR spectroscopy showed that the ratio of the dimer : AIBN was approximately 1:1; if the reaction was heated to reflux for longer than 2 hours, complete degradation of AIBN should occur. But on heating **(128)**, $^{n}Bu_3SnH$ and AIBN overnight on four separate occasion, the yields of product **(129)** were 52, 29, 0 and 0%. Whilst the stannyl hydride reduction of primary thiocarbonates takes longer than secondary thiocarbonates[86], heating to reflux overnight should have been sufficient time to enable reduction, hence the lack of consistent conversion of **(128)** to **(129)** is probably not due to insufficient reaction time.

The amount of AIBN was increased to a greater than stoichiometric amount in four reactions, but yields of **(129)** obtained were only 36, 0, 0 & 0%. Some of the by-products produced were white crystalline needles of indeterminate structure, although the characteristic benzylidene peaks were visible in the ^{1}H NMR spectrum (a singlet at 5.58ppm assigned to 5-H and a multiplet at 7.45ppm due to the aromatic protons); elemental analysis found no tin, but contained a large proportion of nitrogen (9.8-

18.2%) suggesting the products were derived from AIBN. Thus the use of an excess of AIBN was not repeated.



Scheme 2.19: Thermolysis of AIBN and subsequent reactions.

Barton and Motherwell found high temperatures were required to enable primary alkyl radicals to form[87] , thus in an attempt to generate **(140)** more effectively, the reaction temperature was raised. However, upon heating **(128)** with tri-*n*-butyltin hydride and AIBN at a variety of temperatures, no definite pattern can be seen across the range of calidity (table 2.3). Barton and McCombie found that aromatic hydrocarbon solvents enabled the highest yield of products upon stannyl reduction of thiocarbonates[84]. Due to the inconsistency of the reaction using toluene or benzene as solvents, a variety of other solvents were used instead. However table 2.3 indicates hydrocarbon solvents ensured the highest yields, which are the least polar solvents, suggesting that an unstabilised radical was able to react more quickly and form the cyclopentanol **(129)** most efficiently. No highly dilute solvent systems were used, as research of the stannane hydride reduction of cholesterol dithiocarbonates and thiocarbonates by Robins, Wilson and Hansske has shown the absence of concentration effects[88].

Solvent used	Reaction temperature (°C)	Two highest yields of **(129)** (%)
Nitrobenzene	210	0 (& 43% ether **(132)**)
Benzonitrile	188	16, 0
para-Cymene	176-8	0
para-Xylene	138	69, 29
Toluene	111	73, 70
Dioxane	100-102	36
Benzene	86	75, 66
Glyme	85	21 (& 15% **(132)**)
Acetonitrile	82	42, 30 (& 38% **(128)**)
Carbon tetrachloride	77	0 (& 2% **(128)**)
THF	67	0

Table 2.3: Effect of altering temperature and solvent in tin hydride reductions of **(128)**.

Whilst initially only one equivalent of tri-*n*-butyltin hydride was used to reduce each equivalent of thiocarbonate to alkane, in order to determine if the number of equivalents of radical hydride was a factor in the variable results from reduction of **(128)**, the amounts of tin hydride were varied; however, the yield of product did not correlate with the relative quantity of hydrogen radical (table 2.4). The $^{n}Bu_3SnH$ used was obtained from various commercial sources (Aldrich, Avocado, Lancaster chemical companies); in order to determine if the activity of that sourced externally was not sufficient, $^{n}Bu_3SnH$ was made directly from $LiAlH_4$ and tri-*n*-butyltin chloride; whilst the product correlated with the literature[89], upon treatment of **(128)** with the same, the yield of **(129)** was only 41%. Hence it was concluded that the number of equivalents of radical hydride could not be correlated to products, except that using less than one equivalent tended to increase the chance that starting material would be returned.

N° of equivalents of radical	Yields (%) obtained of **(129)**
0.5	54, 30 (& 38% starting material, **(128)**)
0.9	37 (& 8% **(128)**)
1.0	51 (&16% ether **(132)**), 0
1.1	75, 70, 63, 62, 52, 47 (&17% **(132)**), 45, 38, 29, 29, 20, 6, 0, 0, 0; 0 (& 4% **(128)**); 0 (& 2% **(128)**).
1.2	73, 66, 60, 42, 36, 11, 0
1.3	58, 45, 21 (& 15% **(132)**), 0; 0 (& 43% **(132)**)
1.4	0
2.0	40
2.3	41, 29
3.0	0
3.6	0
3.7	70, 16
4.9	69, 45
7.0	41, 0
9.9	31

Table 2.4: Variation in number of equivalents of tin hydride.

Each reductive reaction upon **(128)** was conducted under a nitrogen or argon atmosphere and several techniques were investigated to remove the last traces of oxygen (Table 2.5). However, results did not improve with use of any such de-aeration.

Technique of oxygen removal	Yields (%)
De-gassing under vacuum (≈20mmHg)	58 & 47 (&17% **(132)**)
Bubbling nitrogen through solution	70, 41, 20 & 0
Bubbling argon through solution	45, 29 & 0
Sonication bath	16

Table 2.5: De-aeration methods utilised for radical reduction of **(128)**.

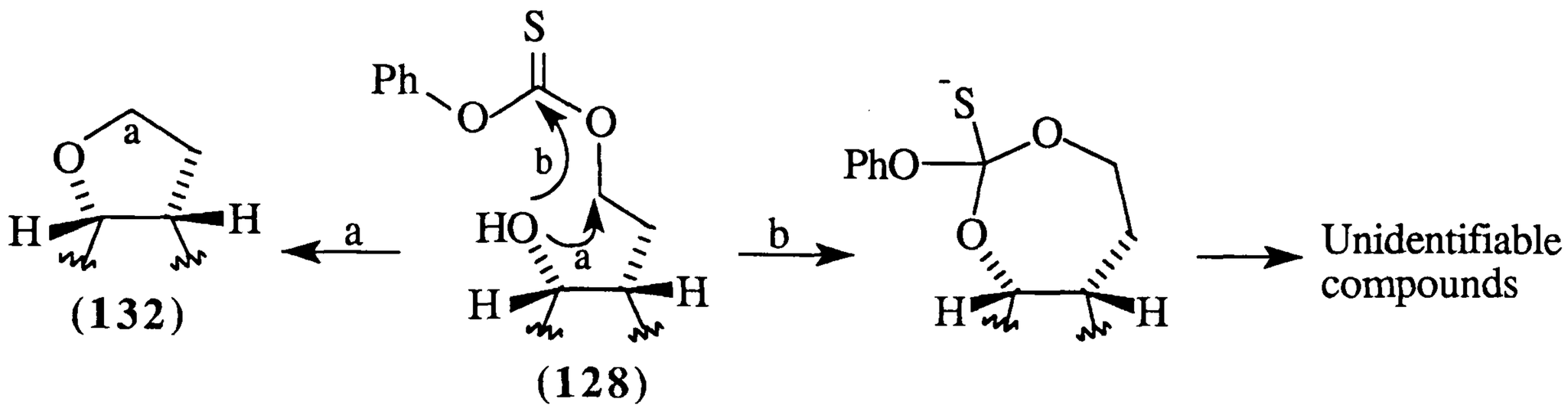
Triphenyltin hydride is an alternative source of hydrogen radicals to the tri-*n*-butyl functionalised molecule, with aromatic rings that can stablise the stannous radical by mesomerism. Whilst cleavage of chloride[90] and carbon-sulphur[91] bonds is more efficient and in higher yield than by tri-*n*-butyltin hydride, little research has been made upon reduction of thiocarbonyl compounds with the Ph_3SnH. Barton and Motherwell found the deoxygenation of a primary xanthate functionality of a hederagenin derivative by triphenyltin hydride less effective than with tri-*n*-butyltin hydride[87]. Treatment of **(128)** with triphenyltin hydride in the presence of AIBN in benzene gave **(129)** in yields of only 47, 26 and 0%.

Triethyl borane was used by Nozaki and co-workers[92] as the radical initiator in a reduction using tri-*n*-butyltin hydride in benzene at room temperature to reduce a secondary phenoxythiocarbonate. This technique was modified for treatment of **(128)** by adding di-*t*-butylpyridine, to prevent degradation of the acetal; however, on use of the borane / hydride reduction system, only starting material (76%) was returned. Repetition of the experiment without di-*t*-butylpyridine gave an unidentifiable mixture of compounds, as did heating tri-*n*-butyltin hydride, di-*t*-butylpyridine, thiocarbonate **(128)** and triethylborane to reflux in benzene for 1.5 hours.

Robinson had used phenylsilane as the hydride source with benzoyl peroxide as the initiator with the phenoxythiocarbonate **(128)** giving only 23-30% yield of cyclopentanol **(129)**[75]. As Barton *et al*[93] had found a greater degree of conversion of thiocarbonates to alkanes with di- and tri- phenylsilane than with phenylsilane, triphenylsilane was used as the hydride source with benzoyl peroxide as the radical initiator, but gave only ≈13% of impure starting material **(128)** after flash chromatographic purification. Treatment of **(128)** with triphenylsilyl hydride and AIBN in toluene heated to reflux returned only starting material (89%). From the results obtained, use of alternative radical initiators to AIBN lowered yields further, as had the use of aromatic silyl reducing agents.

Treatment of **(128)** with tris(trimethylsilyl)silane as the hydride source was proposed, as the silicon-silicon bond enables easy lysis of the weakened silicon-hydrogen bond[94]. Use of tris(trimethylsilyl)silane on the phenoxythiocarbonate **(128)**

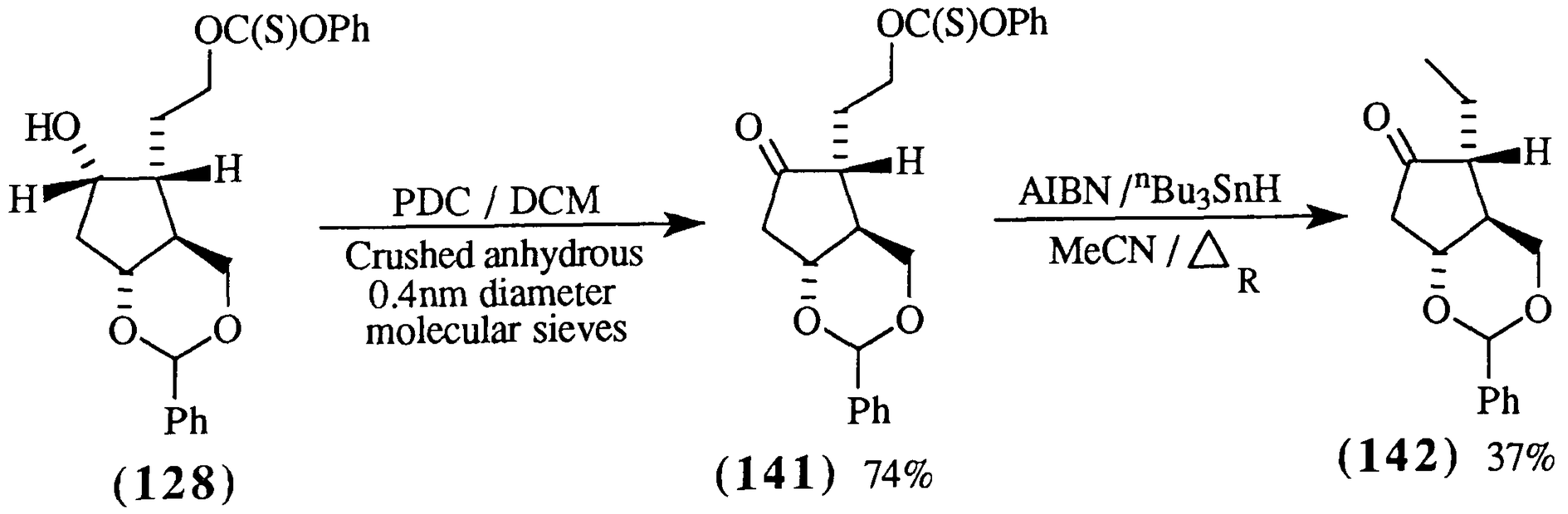
gave odd results. The use of 3.5 equivalents gave a 45% yield of the desired ethyl derivative (**129**). However, using 1.1 equivalents for only 0.5 hours resulted in 65% of (**129**), whilst heating for 2.75 hours gave no isolable components. From these results it was concluded that silylsilanes were no more consistent in reducing the thiocarbonate in (**128**) than stannyl hydride agents.



(**132**) (**128**)

Scheme 2.20: Plausible breakdown pathways of (**128**) to unwanted products.

A possible reason for the poor results of the reduction of (**128**) is the ring alcohol may form a weak van der Waals type linkage to the thiocarbonyl carbon, thereby inhibiting the tin linking to the sulphur; or the free alcohol may enable the cyclisation to the ether (**132**) via the thiocarbonate leaving group (scheme 2.20). Forming the cyclopentanone derivative of the thiocarbonate (**141**) was proposed, as radical reductions do not usually affect ketones[95]. Thus use of PDC and crushed anhydrous 0.4nm diameter molecular sieves in DCM enabled oxidation of (**128**) to give (**141**) in 74% yield, the product being characterised by the presence of both the carbonyl (1699cm^{-1}) and thiocarbonyl (1202cm^{-1}) in the IR spectrum.



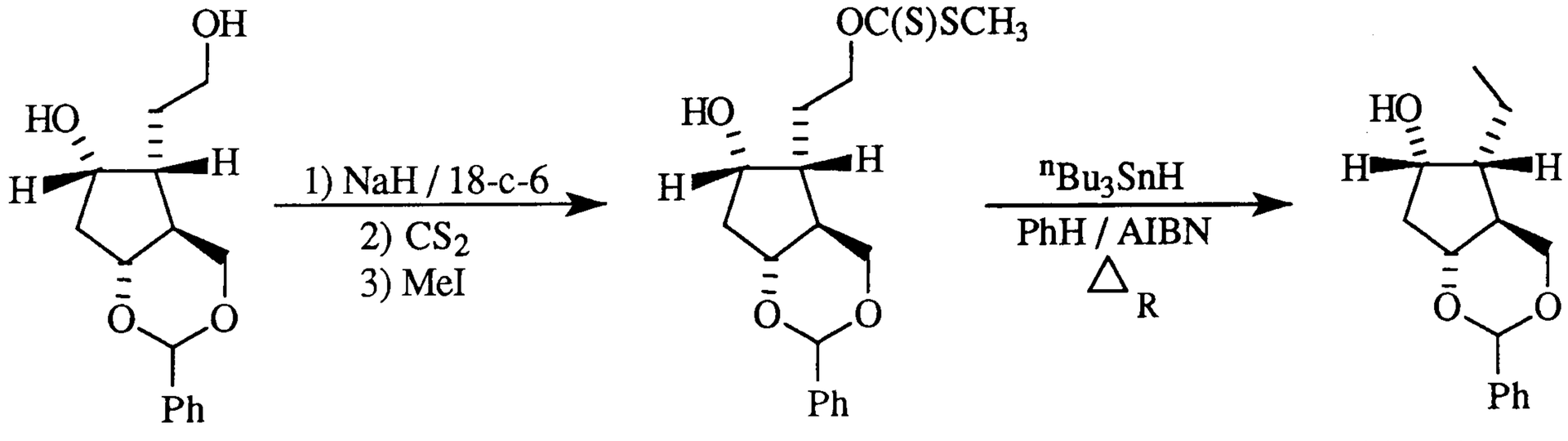
(**128**) (**141**) 74% (**142**) 37%

Scheme 2.21: Synthesis and reduction of keto-phenoxythiocarbonate (**141**).

However, reduction of **(141)** was as erratic as with the 2° alcohol: thrice reacted with tri-*n*-butyltin hydride, yields of the cyclopentanone **(142)** were only 37% (solvent: MeCN), 19% (solvent: PhMe) & 0% (solvent: PhMe).

Several methods for forming an aromatic xanthate derivative[96] of **(127)** were tried, and phenyl chlorodithioformate has the added benefit of being cheaper than phenyl chlorothionocarbonate[97]. Treatment of **(127)** with phenyl chlorodithioformate in the presence of pyridine and / or N-hydroxysuccinimide simply returned starting material. Treatment of **(127)** with potassium or sodium hydride with 18-c-6 prior to addition of phenyl chlorodithioformate merely caused degradation of starting material, as had occurred upon use of metal hydrides with **(127)** and phenyl chlorothionoformate.

Another method for forming a xanthate is to use carbon disulphide, methyl iodide and base[84]. Under these conditions the crude product **(143)** was obtained in 94% yield, the signal in the ^{13}C NMR spectrum at 216ppm being in the characteristic region of a dithiocarbonate. Radical reduction of **(143)** gave the desired cyclopentanol **(129)** in 40% yield with tri-*n*-butyl tin hydride and in 43% yield with triphenyl tin hydride. On purification of the xanthate **(143)** prior to reduction, **(129)** was obtained in only 12% yield. An attempt to use the crude phenoxythiocarbonate **(128)**, i.e. without column chromatography, in a fashion analogous to that for the methyl xanthate **(143)**, in a radical reduction yielded only 4% of the cyclopentanol **(129)**, so was abandoned.



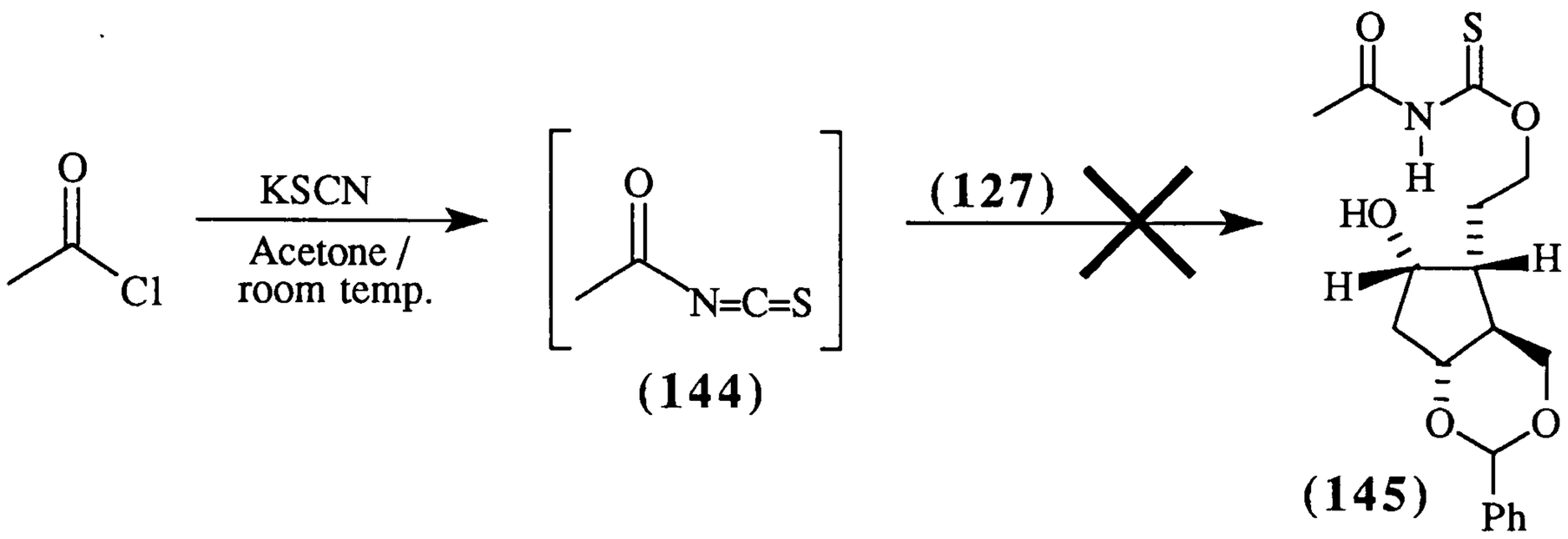
(127) **(143)** 37% **(129)** 40%

Scheme 2.22: Path for deoxygenation of diol **(127)** via xanthate **(143)**.

Although the crude methyl xanthate **(143)** had formed in good yield and had not required purification by column chromatography to enable reduction by tin hydride reducing agents, the yields of **(129)** were not a vast improvement upon radical reductive treatment of **(128)**; the use of carbon disulfide was not a reagent of choice, hence the

use of the less malodorous phenyl chorothionoformate as thiocarbonate derivatisation agent was retained.

A further radical deoxygenation procedure was attempted based on the work of Oba and Nishiyama[98], *via* reacting the proposed *N*-acyl thioxocarbamate (**145**) with tri-*n*-butyltin hydride with AIBN in benzene. The oxoisothiocyanate (**144**), which is not isolated in the literature procedure, was presumably formed and the alcohol (**127**) was added to the oxoisothiocyanate and heated to reflux overnight in a 'one-pot' reaction; however, as indicated none of the thioxocarbamate (**145**) could be isolated, and only the diol (**127**) was identified in 5% yield (scheme 2.23).

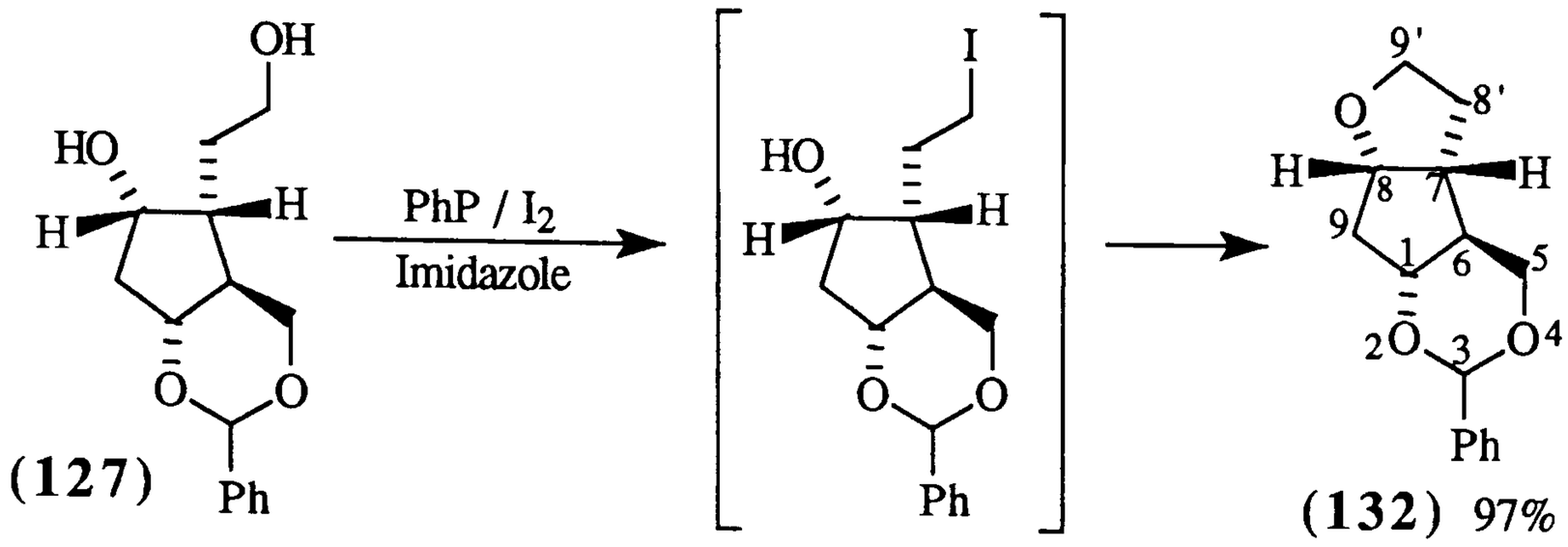


Scheme 2.23: Proposed route to form *N*-acyl thioxocarbamate from diol (**127**).

Thus a variety of radical hydride sources, initiators and thiocarbonyl derivatives were employed in many attempts to reduce a primary alcohol to an ethyl group, to form C(16) and C(17) of the target mycinolide; treatment of the phenoxythiocarbonate (**128**) with tri-*n*-butyltin hydride and AIBN in heated aromatic hydrocarbon solvents was inconsistent, but did return product (**129**) in widely varying yields.

Instead of reduction of a thiocarbonate derivative, the use of a halide was explored, also to be deoxygenated by a radical hydride source. Treatment of the diol (**127**) with triphenylphosphine, iodine and imidazole[99] in an attempt to form the primary iodide gave the cyclised ether (**132**) in 97% yield (scheme 2.24). The primary iodide probably did form under these conditions, but was displaced by the secondary alcohol. The ^{1}H NMR spectrum of (**132**) was characterised by the collapse of the signals at 3.65ppm (dt, J=10 and 2.5Hz, 9'-H) and 3.89ppm (dt, J=10 and 4Hz, 9'-H)

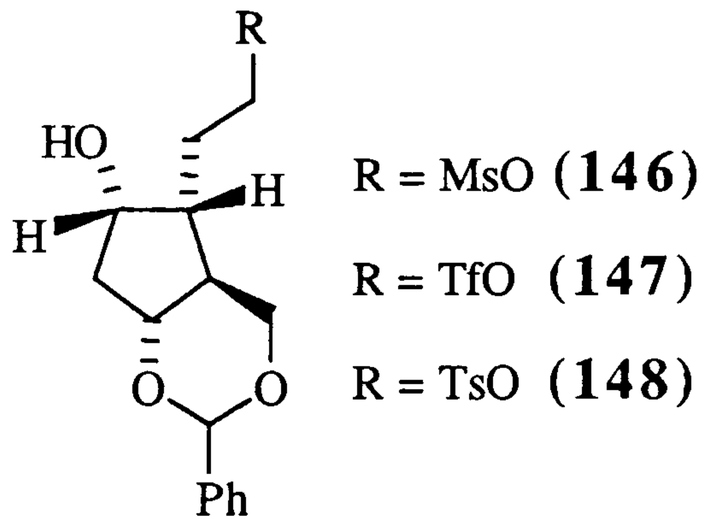
due to the protons α to the primary alcohol of **(127)** and the appearance of a large multiplet at 3.89ppm, assigned to the two protons at 9' of the ether **(132)**.



(127) **(132)** 97%

Scheme 2.24: Attempted formation of the primary iodide.

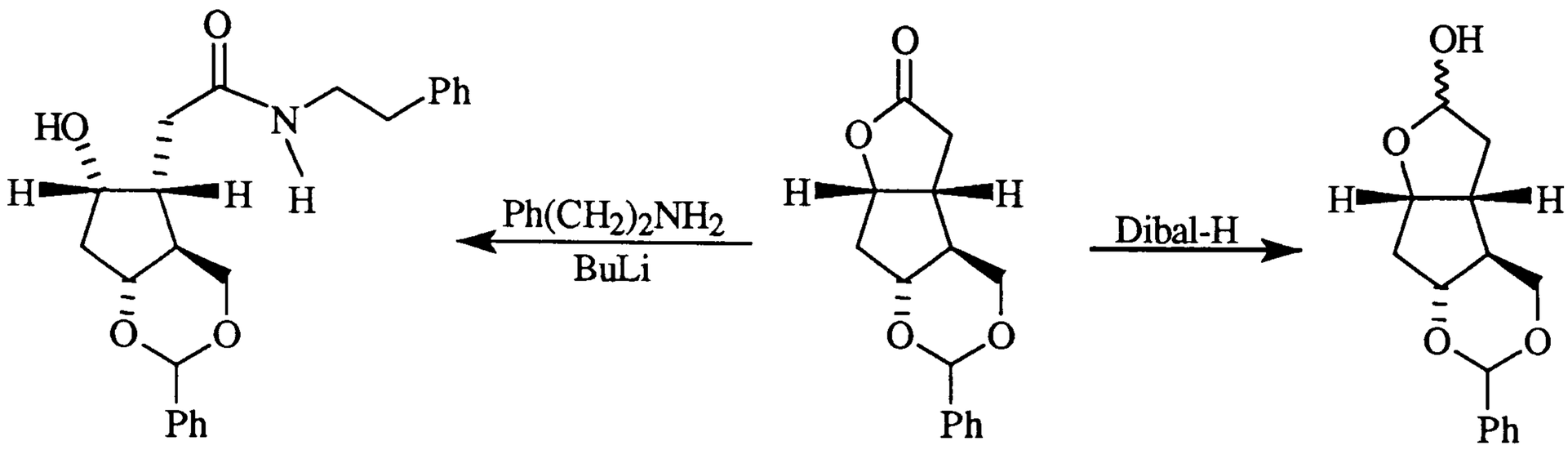
The methanesulfonate **(146)**, trifluoromethanesulfonate **(147)** or *para*-toluenesulfonate **(148)** were other derivatives which were considered since both could, in theory, be reduced with metal hydrides[100]. However on treatment of **(127)** with a variety of sulfonyl reagents (MsCl, MsBr, Ms_2O, TsCl, TsBr and TfCl) and bases (Et_3N, DMAP, pyridine and NaH) in each case gave a mixture of unidentifiable products or on occasions the cyclic ether **(132)**.



The use of trimethylsilyl chloride (after reacting the primary alcohol **(127)** with tosyl chloride in the presence of of DMAP and three equivalents of pyridine) to protect the secondary alcohol and prevent cyclising to **(132)** was attempted; however, the secondary alcohol was not protected as the yield of the cyclic ether was 73%.

Due to the disappointing results from selective reduction of the primary alcohol in **(127)** to **(129)**, an alternative approach was examined involving reduction of a derivative of the corresponding lactol / hydroxy aldehyde **(149)** (scheme 2.25). Reduction of the lactone **(126)** with Dibal-H gave **(149)** in 74% yield (plus 11% starting

material). Lactol (**149**) was characterised by the absence of a carbonyl moiety by either IR or ^{13}C NMR spectra. A Huang-Minlon[101] modified Wolff-Kishner reaction upon the lactol (**149**) was undertaken but gave no isolated compounds upon work-up, which may be due to the presence of strong base at high temperature degrading the molecules. The literature notes that ketones are more stable to such hydrazine based reductions, and the aldehydes listed that had been successfully reduced were either conjugated or aromatic[102].



(**150**) 59% (**126**) (**149**) 74%

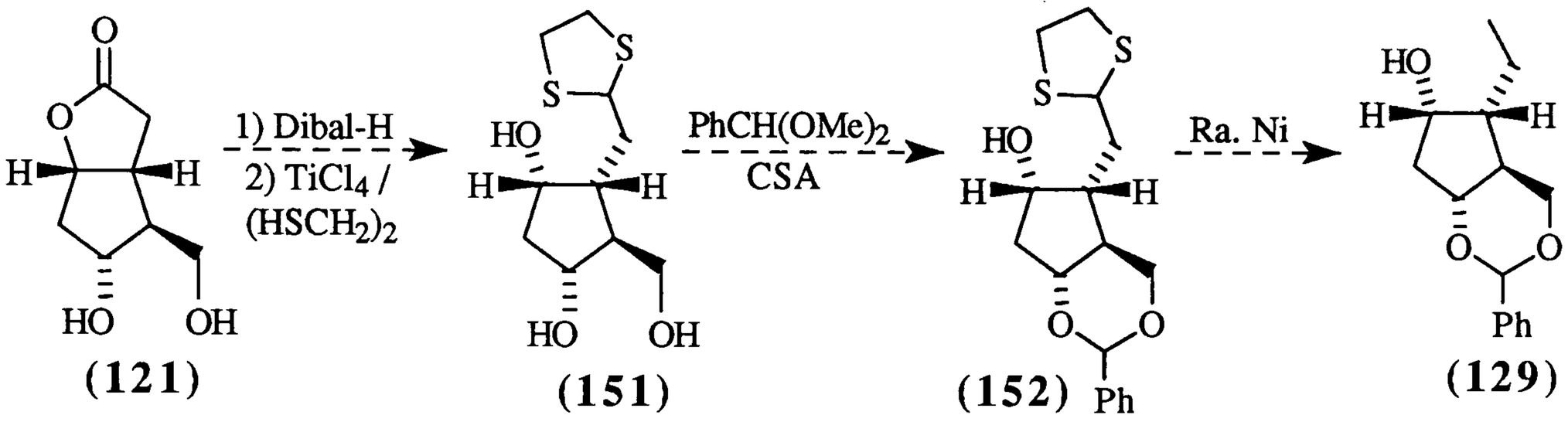
Scheme 2.25: Treatment of (**126**) to form hydroxy-carbonyl compounds.

A modification of the Wolff-Kishner reaction is the treatment of a carbonyl with tosylhydrazine and catalytic tosic acid to produce a tosylhydrazone, which may be reduced to an alkane with a mild hydride source such as sodium cyanoborohydride[103]. Thus lactol (**149**) was stirred with 1.2 equivalents of tosylhydrazine (to prevent degradation of the acetal) and 0.2 equivalents of tosic acid in an attempt to form the hydrazone, which was to be reacted with a silyl group to protect the secondary alcohol, followed by reaction with sodium cyanoborohydride and final deprotection of the alcohol with TBAF. However, the hydrazone did not form, but 83% of the starting material was returned.

As the hydrazones of lactol (**149**) could not be formed, it was proposed that a highly nucleophilic amine anion would open the γ-lactone ring of (**126**) to form an amide, which after reduction may be able to undergo a Hoffman elimination (scheme 2.26). Treatment of β-phenylethylamine with butyl lithium at -78°C gave an anion, which upon addition to lactone (**126**) formed the amide (**150**) in 59% yield; the carbonyl signal in the IR spectra was 1617cm^{-1}, a value characteristic for a saturated

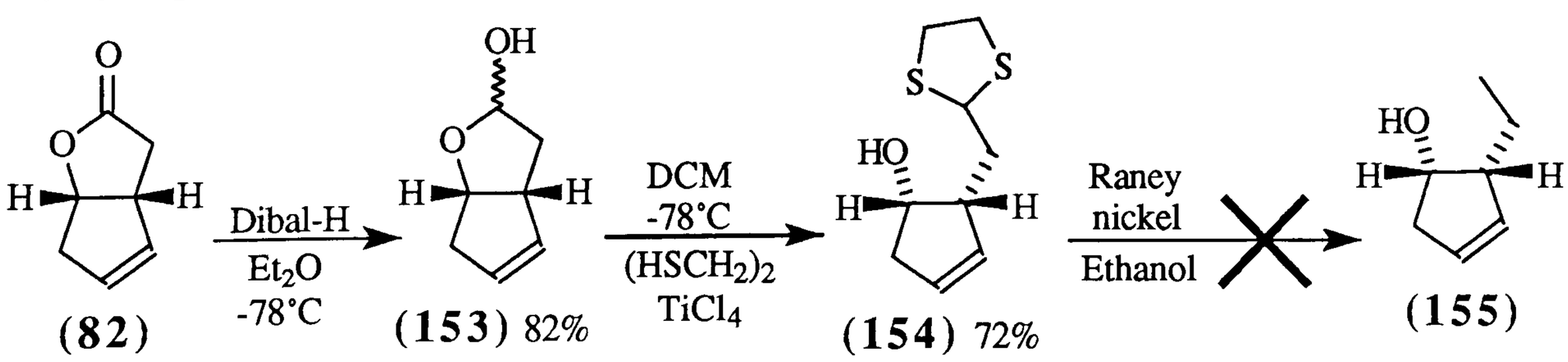
amide. However, on addition of lithium aluminium hydride to the amide, a complex mixture of products was formed, from which none of the required amine could be identified. Attempted protection of the secondary alcohol in **(150)** simply returned starting material.

Another method which was considered was reduction of a dithiane **(152)** with Raney® nickel[104]. The dithiane could not have been added to upon the benzylidene protected γ-lactol **(149)** directly, because the strongly acidic conditions would degrade the benzylidene group. The great polarity of the diol **(121)** made handling difficult, hence this approach was investigated on a model system (scheme 2.27).



Scheme 2.26: Proposed route to cyclopentanol **(129)** from diol **(121).**

Lactone **(82)** was reduced by one equivalent of Dibal-H to form the lactol **(153)** as a mixture of epimers. Reaction of the masked aldehyde functionality of **(153)** with ethanedithiol in the presence of titanium tetrachloride as a Lewis acid catalyst gave the dithiane **(154)** in 72% yield. However, on treatment of **(154)** with commercially obtained Raney Nickel[97] in anhydrous methanol or ethanol, none of the desired ethylcyclopentenol **(155)** was seen, but starting material was returned reproducibly.

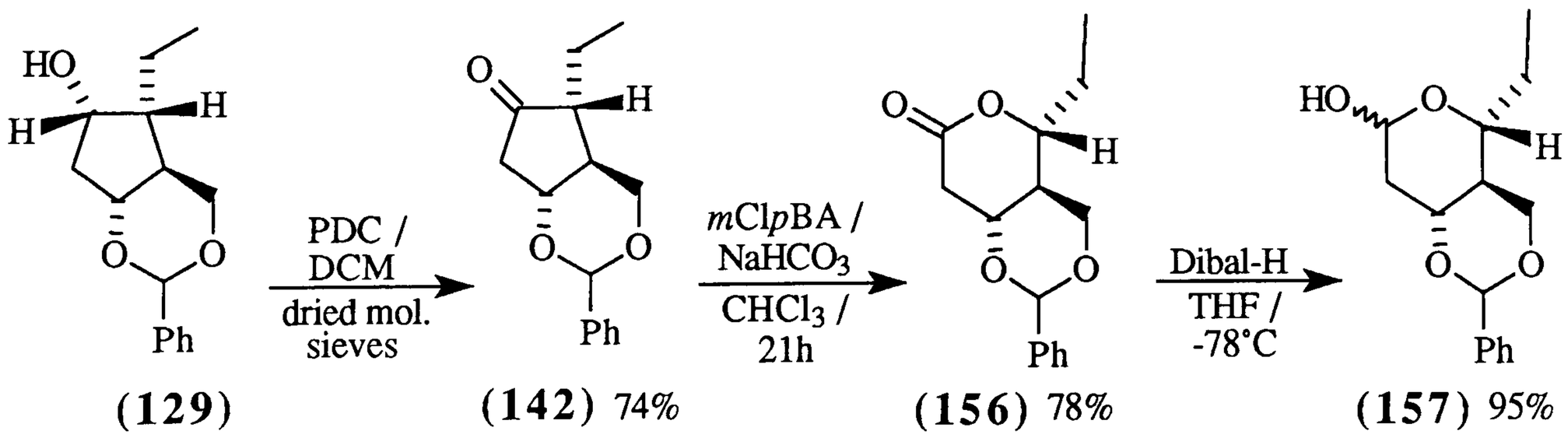


Scheme 2.27: Model study of an attempted reduction of lactone **(82)**.

As the several pathways to obtaining the desired ethyl group for positions C(16) and C(17) of the target mycinolide by avoiding the necessity to use radical deoxygenations had failed, the use of tri-*n*-butyltin hydride upon phenoxythiocarbonate **(128)** in an aromatic hydrocarbon solvent in the presence of AIBN as the radical initiator was used in the synthesis of the desired ethyl group of **(129)**. The most logical reason for the lack of consistent reduction is that the route generates a primary radical, which is not as stable as a secondary or tertiary radical[105]. However, Freidline and co-workers[106] noted "In the case of less stable (secondary, primary) radicals, fragmentation competes with hydrogen abstraction from Bu_3SnH by the radical [**(136)**]". The secondary alcohol at C-8 of the cyclopentanol **(129)** also could cyclise to enable formation of the tetrahydrofuran acetal **(132)** (or form unidentifiable products), and the secondary alcohol proved difficult to protect in order to prevent reaction with substituents on the primary alcohol.

2.3.3 Studies Towards the Synthesis of Fragment A from **(129)**

The next 3 steps in the synthesis of fragment A of mycinamicin III and mycinoic acid II proceeded smoothly following similar procedues to those used by Robinson (Scheme 2.28)[75]. Oxidation of cyclopentanol **(129)** using PDC and anhydrous crushed sieves in DCM gave the required ketone **(142)** in 74% yield with no apparent epimerisation α to the carbonyl group. Other methods were briefly examined to see if a higher yield of the ketone **(142)** could be obtained.

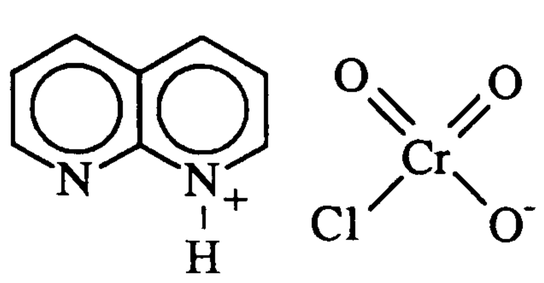


(129) **(142)** 74% **(156)** 78% **(157)** 95%

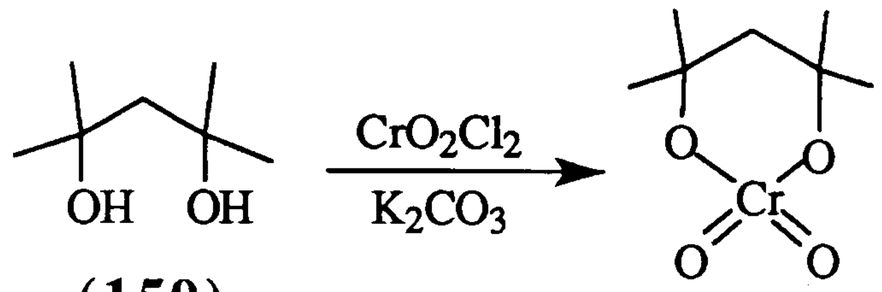
Scheme 2.28: Synthesis to δ-lactol **(157)**.

Treatment of cyclopentanol **(129)** with anhydrous pyridinium chlorochromate (PCC) in the presence of dried sodium hydrogen carbonate (to prevent loss of the

benzylidene protecting group) gave only 30% of ketone (**142**). A related oxidation method to that of PCC was the use of 2,2'-bipyridinium chlorochromate[107] (**158**), which has a second heterocyclic nitrogen which can prevent the acidic by-products degrading the benzylidene group. This enabled oxidation of (**129**) to give (**142**) in a yield of 70% which was not an improvement upon use of PDC with molecular sieves, and was not significantly faster either, as the bipyridinium-based reaction required stirring for 2.75 hours to enable all starting material to disappear by TLC analysis.



(**158**)



(**159**) (**160**)

Figure 2.3: Oxidants used to convert cyclopentanol (**129**) to cyclopentanone (**142**).

An alkyl chromate oxidant (**160**) was first used in catalytic quantity by Corey *et al*[108] with a stoichiometric amount of peracetic acid to effect oxidation of secondary alcohols to ketones. A modification of this method by Luzzio and Moore[109] was to effect oxidation with non-purifed (**160**), which has titanium dioxide and potassium carbonate still present; Morin-Fox and Lipton[110] utilized the alkyl chromate with concurrent use of four equivalents of *m*Cl*p*BA as co-oxidant to oxidise secondary alcohols directly to lactones. Treatment of cyclopentanol (**129**) according to the method of Luzzio and Moore with four equivalents of non-purified (**160**) gave the ketone (**142**) in 72% yield, but even with stirring for approximately 96 hours, none of the cyclopentanone was converted to δ-lactone (**156**); also the reaction took almost 24 hours to form the ketone, which is far longer than the ionic chromate agents.

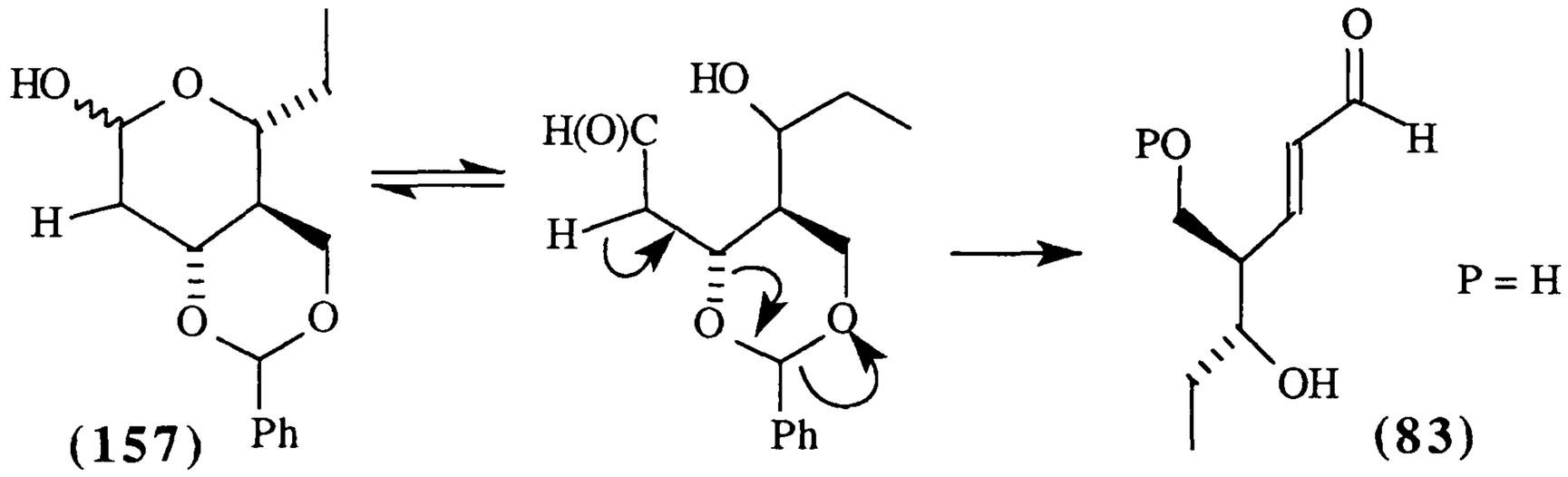
Sulfur trioxide pyridine complex in DMSO can oxidise alcohols readily (the Jekishan modification of the Moffat oxidation) to aldehydes or ketones and the oxidants give easily removable by-products once reacted[111]. Treatment of (**129**) with pyridine sulfur trioxide, in the presence of triethylamine to prevent breakdown of the acetal, returned the ketone (**142**) from alcohol (**129**) in 64% yield, but the product was not as pure as that from chromate oxidations, and it was necessary to remove DMSO under high vacuum. Use of TPAP, and NMO as co-oxidant[112], to oxidise cyclopentanol (**129**)

gave the ketone (**142**) but the yield obtained was only 62% and the reaction was no faster than with PDC and molecular sieves.

Considering the efficacy, cost, convenience and speed of of the reagents used in the oxidation of (**129**) to (**142**), none were an improvement upon the use of PDC and crushed 0.4nm diameter anhydrous molecular sieves in a chlorinated solvent, which was the reagent of choice.

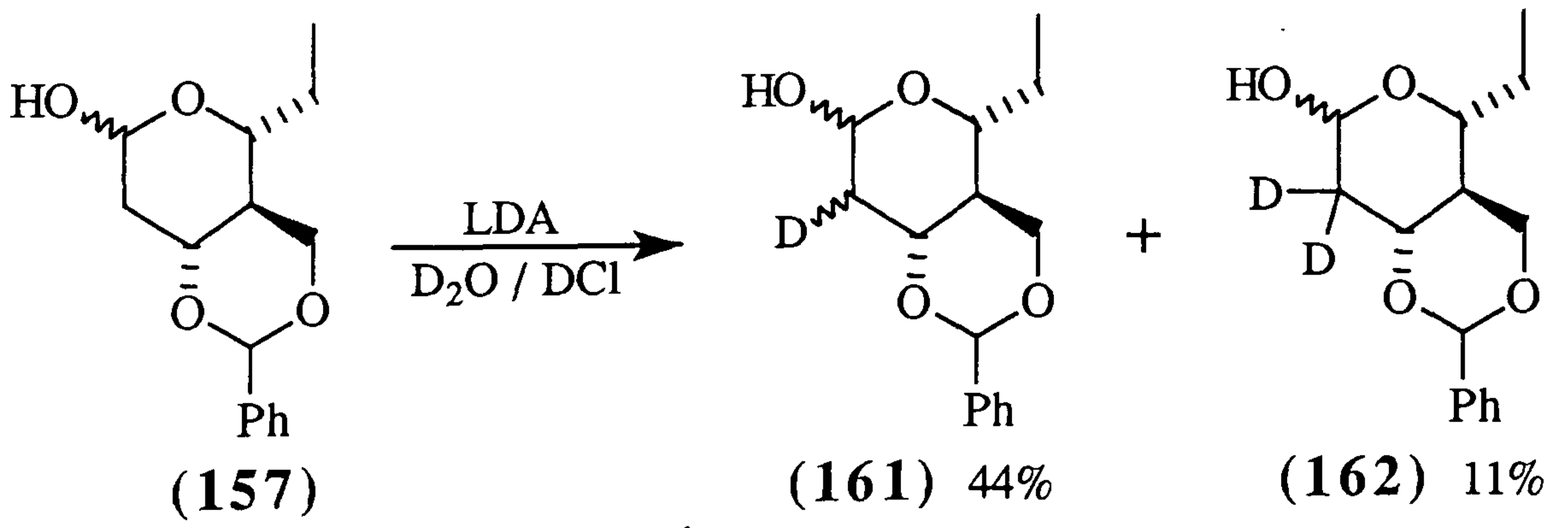
Treatment of ketone (**142**) with *m*Cl*p*BA / $NaHCO_{3(s)}$ gave the required δ-lactone (**156**) with complete regio- and stereo-control in 78% yield (scheme 2.28). Reduction of the lactone (**156**) to the δ-lactol (**157**) with Dibal-H also proceeded smoothly in over 90% on virtually all occasions.

With quantities of the lactol (**157**) in hand, to complete the synthesis of fragment A of mycinolide III it was simply necessary to effect a β-elimination of the benzylidene group giving (**83**).



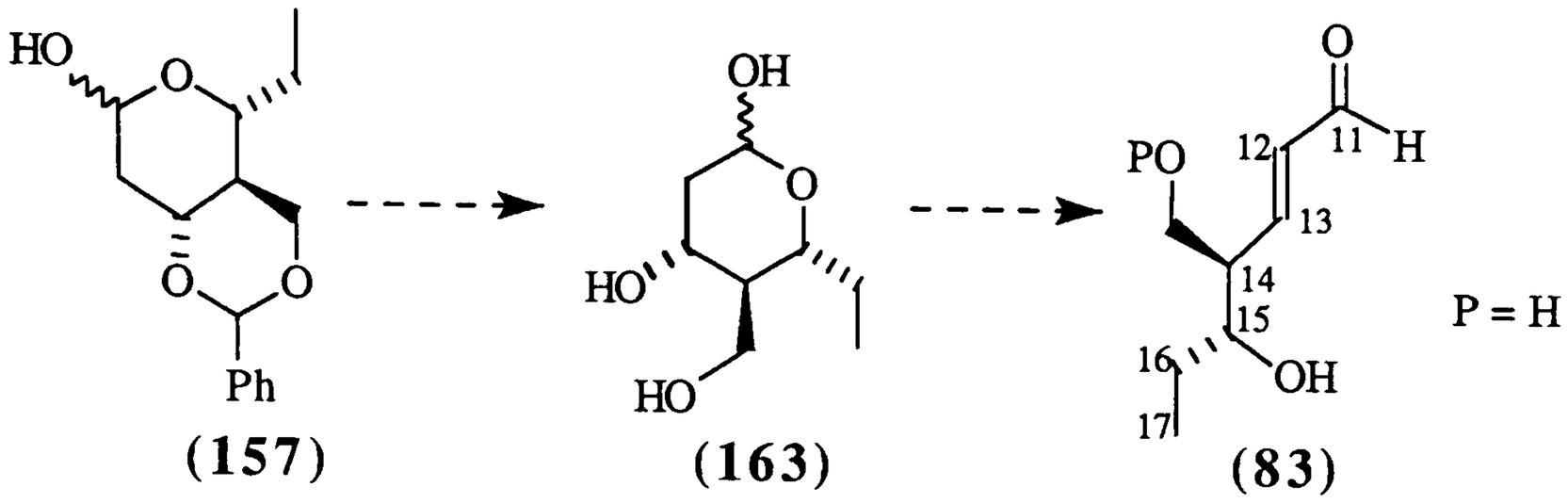
Scheme 2.29: Proposed mechanism to give fragment A (**83**).

Robinson[75] had previously shown that reaction of (**157**) with LDA returned starting material. Addition of D_2O to the reaction mixture confirmed that the enolate had indeed been formed, but surprisingly no β-elimination had occurred.



Scheme 2.30: Deuterium incorporation into lactols (**157**)[75].

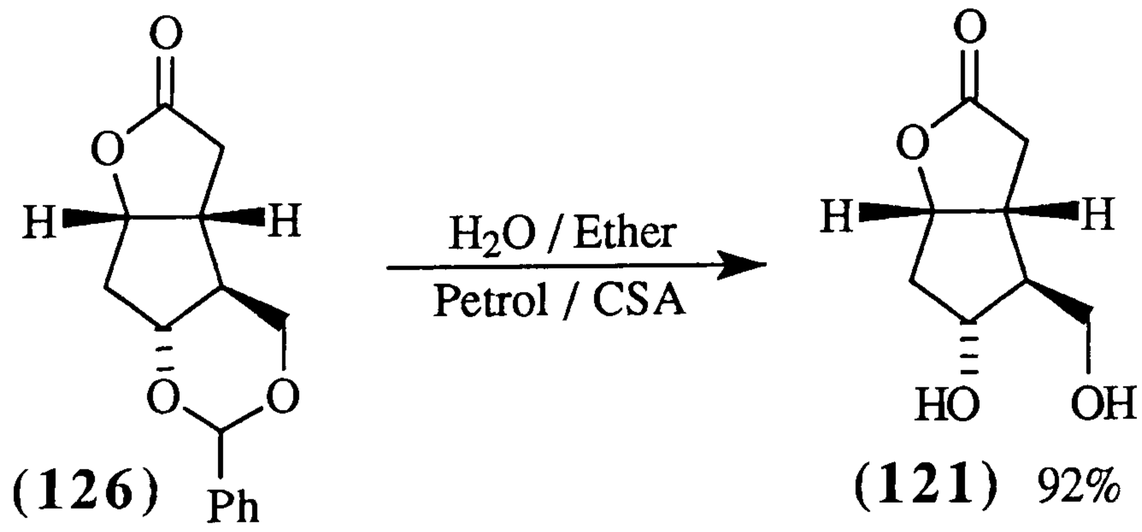
Therefore it was deemed necessary to remove the benzylidene group before elimination to the required α,β-unsaturated aldehyde **(83)**.



Scheme 2.31: Proposed route to **(83)** via free hydroxyl derivative **(158)**.

Several methods were examined to convert **(157)** to **(163)**. Reaction of the lactol with methanol and acidic Dowex® resin followed by an acidic work-up gave a complex mixture of products. Attempted deprotection of **(157)** by using either acidic Dowex® resin in anhydrous DMSO, or dilute hydrochloric acid and THF returned only starting material in low yield after an alkali extraction and work-up. Thus other methods of removal of the benzylidene acetal to give either the α,β-unsaturated aldehyde **(83)** or the tri-hydroxy precursor **(163)** were proposed.

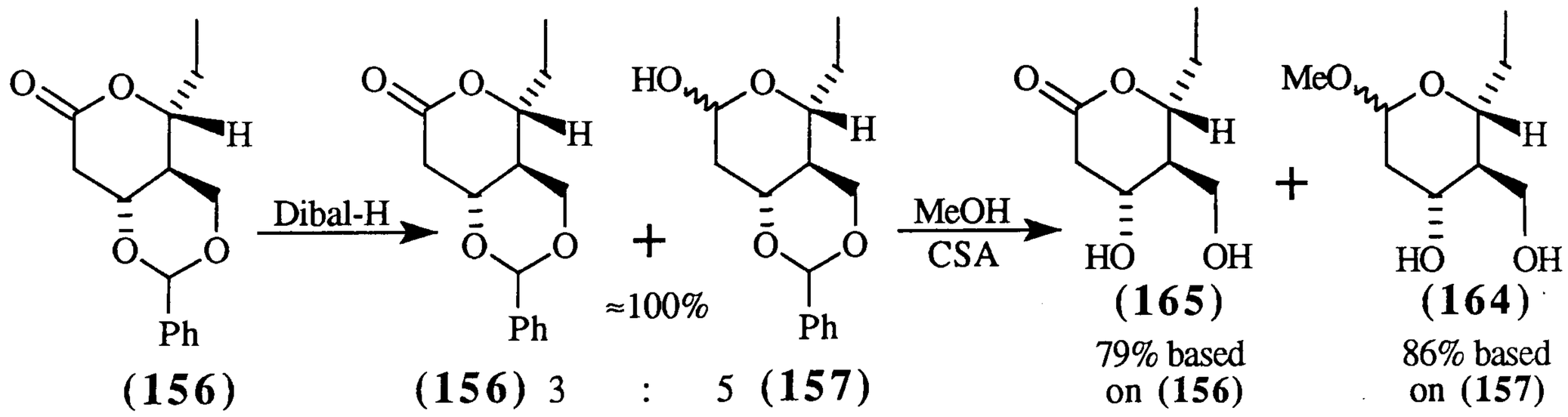
The dihydroxy lactol **(163)** was predicted to be very polar and hence difficult to extract into organic solvents, thus the use of a biphasic system consisting of an upper organic layer and a lower aqueous acidic layer was considered. As the benzylidene is cleaved, it was proposed that the resulting benzaldehyde should pass into the organic solvent, and the diol remains in the aqueous layer and could be recovered by removal of the water. A model study on an acetal **(126)** using a mixture of petrol, ether and CSA dissolved in water returned the diol **(121)** in 92% yield from the aqueous portion after evaporation of water and purification by column chromatography.



Scheme 2.32: Model study: removal of benzylidene group of **(126)**.

Treatment of **(157)** under similar conditions gave ≈40% return of starting material but no other compounds were characterised. Stirring **(157)** in petrol, methanol and water with *para*-toluenesulfonic acid gave the methoxy acetal **(164)** in 45% yield, which was readily discernible by the appearance of singlets at 3.30 and 3.49ppm, in a ratio of 3:1, due to the methoxy groups of the two isomers; no other components of the reaction were characterised. Treatment of **(157)** with *para*-toluenesulfonic acid in a biphasic system of petrol and water resulted in the return of no identifiable components.

On one occassion, treatment of **(156)** with Dibal-H did not completely reduce the lactone to the lactol, but gave a mixture of **(156)** and **(157)** (in a ratio of 3:5) which could not be separated by column chromatography. Interestingly, treatment of the mixture with anhydrous methanol and CSA gave **(164)** and **(165)** (scheme 2.33), which were extracted from an alkaline work-up procedure and the diols separated by column chromatography. This yield of the methoxy acetal **(164)** was much higher than that obtained when **(157)** was stirred in petrol, methanol and water with *para*-toluenesulfonic acid, which only gave **(164)** in 45% yield; this suggests the anhydrous conditions prevented some of the decay of the acetal functionality.



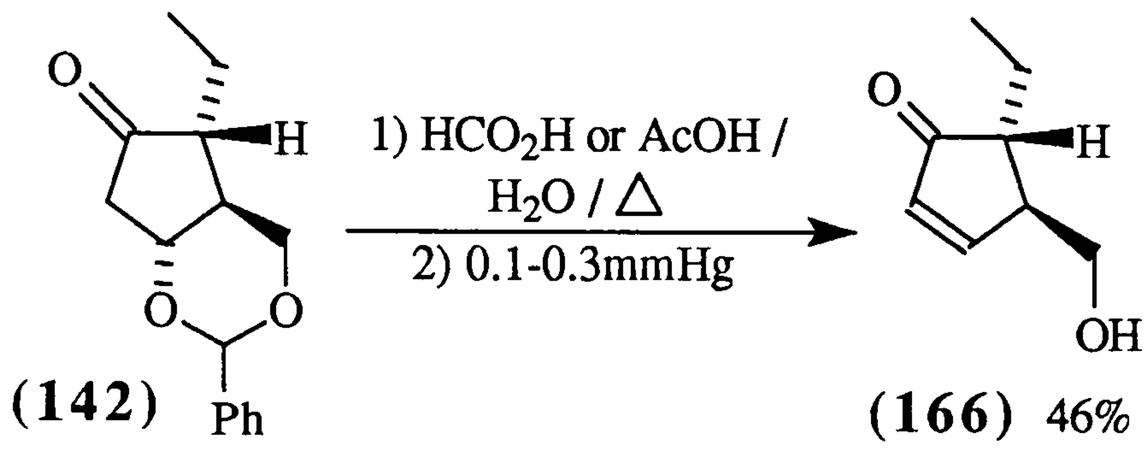
Scheme 2.33: Removal of benzylidene protecting group.

Dibal-H reduction of **(165)** should lead to the masked aldehyde **(163)**; however repeated attempts to reduce **(165)** with three equivalents of Dibal-H gave a complex mixture of products.

With a small amount of the dihydroxy acetal **(164)** available, deprotection of the acetal and elimination to the α,β-unsaturated aldehyde was examined. Treatment of **(164)** with CSA, water and petrol in a biphasic system gave no identifiable products and

a similar result was obtained when **(164)** was heated to 66°C in water under vacuum (≈20mmHg) with acidic Dowex.

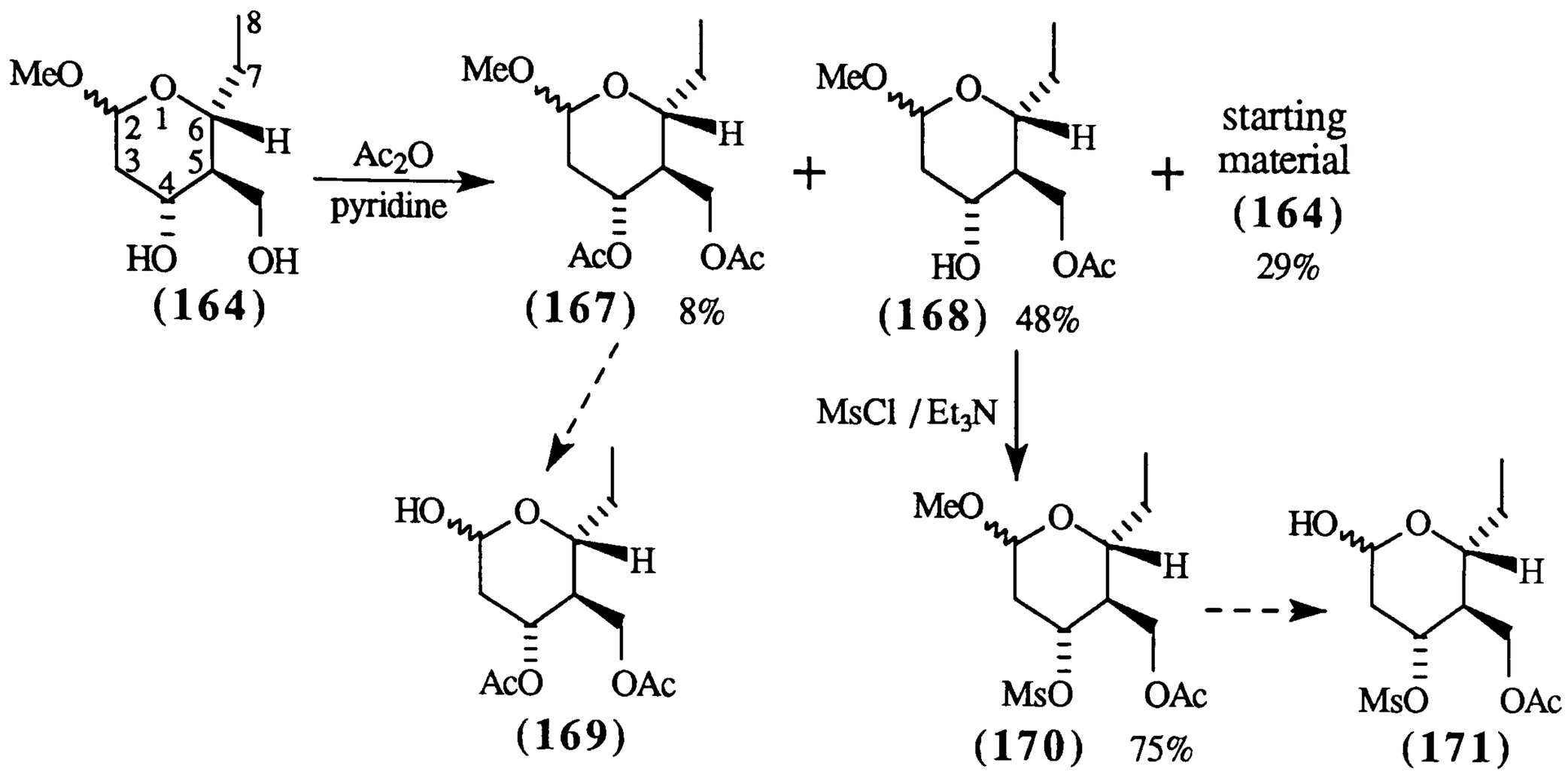
Another approach which was examined to enable production of the dihydroxy lactol **(163)** was to remove the acetal protecting group prior to the Baeyer Villiger reaction on the substituted cyclopentanone **(142)**. However on heating **(142)** with formic acid in water or with acetic acid, followed by removal of the acid under vacuum formed the unsaturated ketone **(166)** in 46% yield (with HCO_2H) and 41% yield (with CH_3CO_2H) (scheme 2.35). Interestingly treating the corresponding δ-lactone **(156)** under similar conditions simply returned starting material.



(142) **(166)** 46%

Scheme 2.34: One-pot deprotection and elimination from ketone **(142)**.

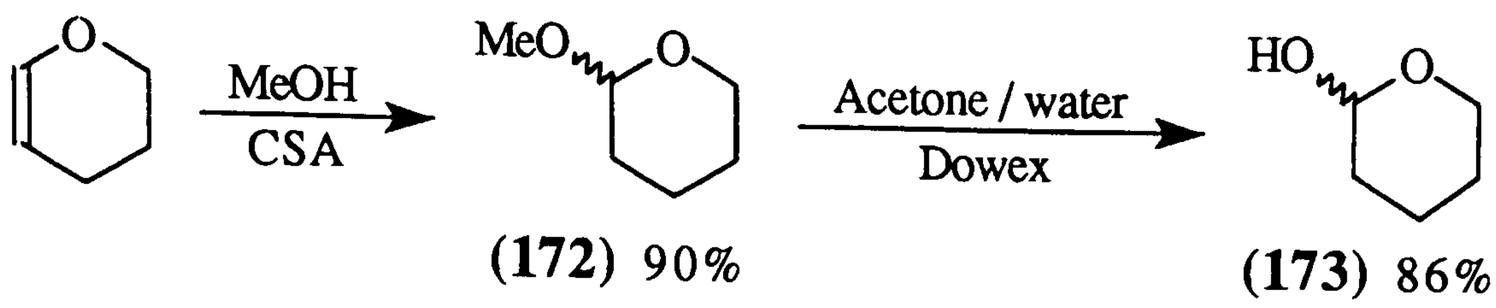
It was apparent that neither the alcohol nor the benzylidene derivative at C(3) was a good enough leaving group to form the required α,β-unsaturated aldehyde, thus it was proposed to use a better leaving group e.g. an acetate or mesylate (scheme 2.35). Treatment of diol **(164)** with 1 equivalent of acetic anhydride and pyridine gave a mixture of the C(2) epimers of the diacetate **(167)**, the required monoacetate **(168)** and starting material. The products were separated by column chromatography. By analysis of the ^1H NMR spectra of the more prevalent epimer of the monoacetate **(168)**, the monoacetate was identified by the appearance of a multiplet at 3.85ppm which is not visible in the spectra of the diacetate **(167)**; the diacetate has a peak (not visible in the spectra of **(168)**) at 5.19ppm due to the proton α to the ring acetate at C(4) (J=12.5 and 5Hz).



Scheme 2.35: Attempted eliminations of methoxy acetals.

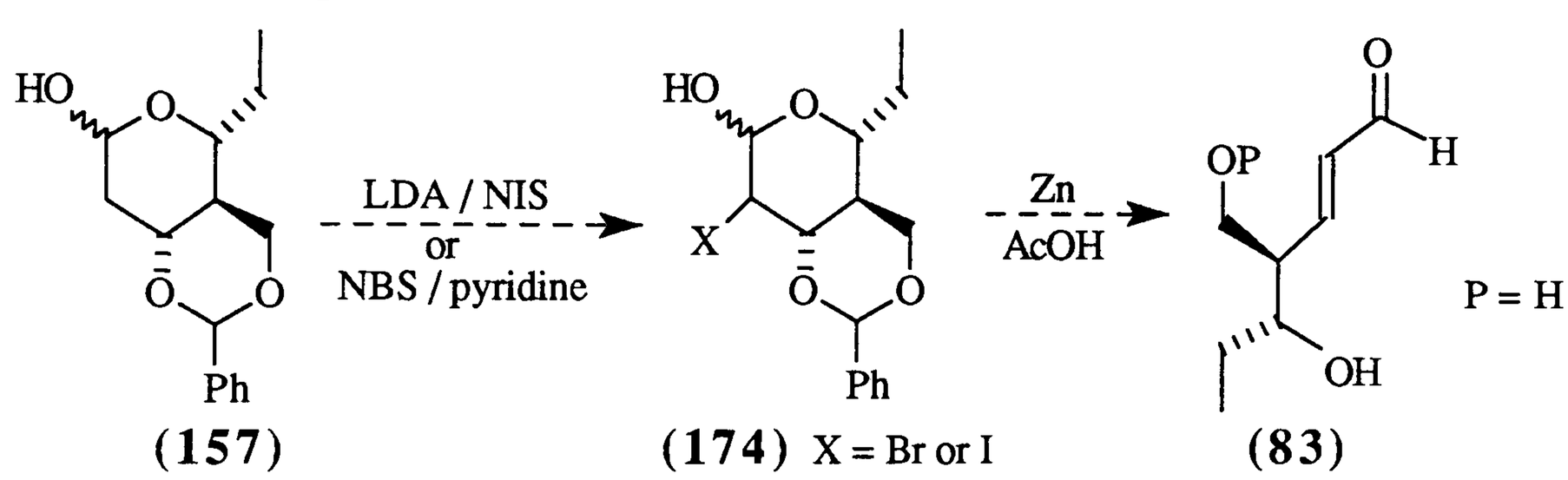
Monoacetate (**168**) was treated with methanesulfonyl chloride in the presence of triethylamine to give the methanesulfonate (**170**) in 75% yield. Repeated attempts to deprotect the acetal in either (**167**) or (**170**) failed to give the lactols (**169**) or (**171**) for the final elimination to the unsaturated aldehyde (**83**). For example, on treatment of (**170**) with dilute hydrochloric acid in acetonitrile, or acidic Dowex in acetone and water, or formaldehyde with $TiCl_4$ in THF, or dilute hydrochloric acid in THF, or pyridine and DMAP in DCM, starting material was returned in varying proportions. Likewise, the diacetate (**167**) was returned in 30% yield only from an attempted elimination with dilute hydrochloric acid in ether, and approximately quantitatively from a media of acidic Dowex resin in acetone; however, the use of DMAP and pyridine destroyed the diacetate (**167**) and nothing was identifiable on extraction.

Due to the repeated failure to remove the methoxy acetal, further methods were investigated on a model system. Thus dihydropyran was reacted with CSA in methanol and upon purification gave the methoxy acetal (**172**); subsequent reaction with acidic Dowex in a mixture of acetone and water gave the δ-lactol (**173**) (scheme 2.36). Thus the reason the diacetate (**167**) and methanesulfonate (**170**) acetal functionalities failed to be removed is unclear, as the steric effects of groups β and γ to the acetal carbon of both (**167**) and (**170**) do not appear to be a problem.

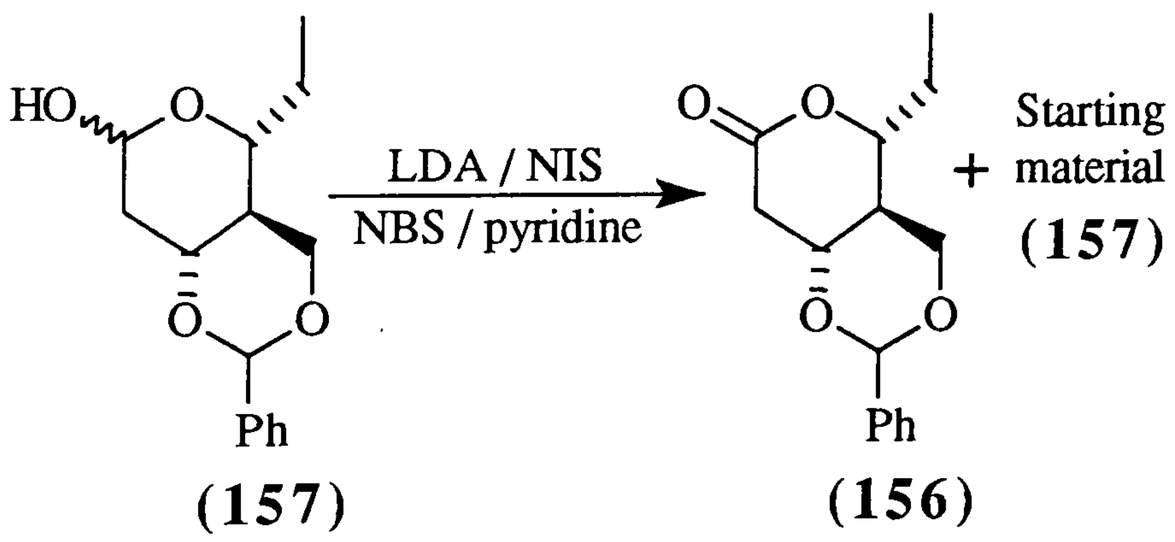


Scheme 2.36: Removal of methoxy acetal from a model.

Due to the lack of success using diols **(164)** or **(165)** as intermediates in the synthesis of fragment A of mycinolide III, an attempt was made to add a halogen (either Br or I) α to the masked carbonyl of lactol **(157)**. Reduction and elimination of the halide from **(174)** with zinc in a manner not dissimilar to that of dichloroketone **(86)**, should give the unsaturated aldehyde **(83)**. However, treatment of lactol **(157)** with either LDA and NIS or pyridine and NBS led to oxidation of the lactol to the lactone **(156)**, no incorporation of a halogen was apparent by NMR or mass spectrometry. When the reaction was repeated under acidic conditions with iodine and acetic acid a complex mixture of products was formed.



Scheme 2.37: Proposed zinc / halide reductive route to fragment A.



Scheme 2.38: Oxidation of lactol functionality of **(157)**.

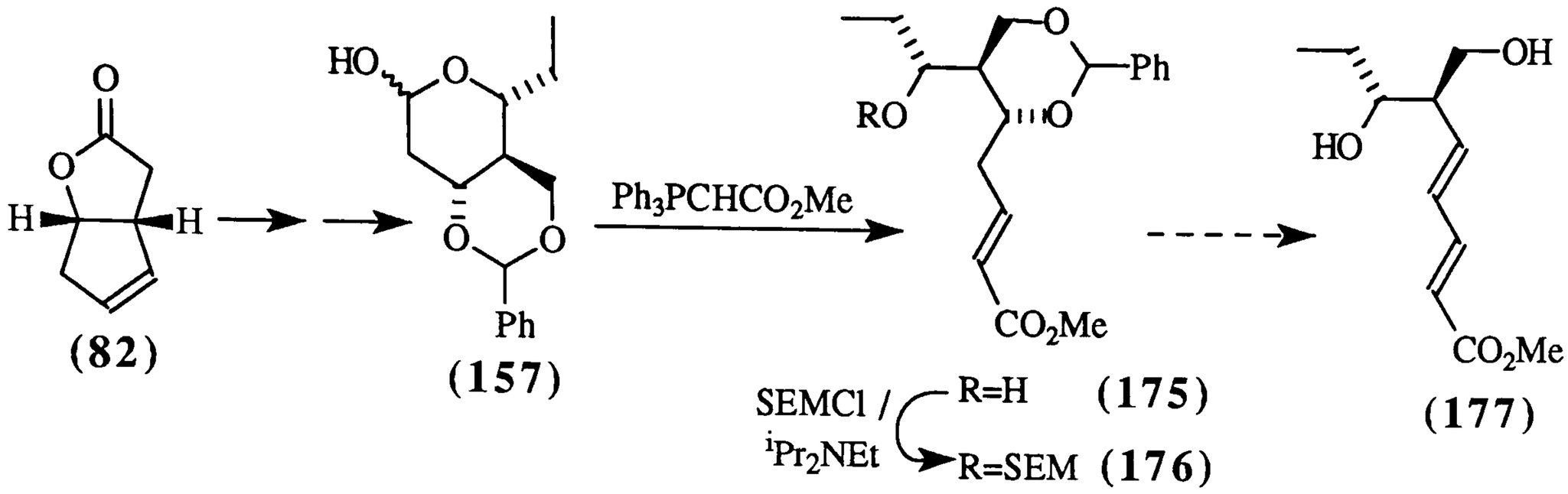
In an novel approach to obtain **(163)**, the α,β-unsaturated ester **(178)** (derived from **(157)** in studies towards mycinoic acid II, section 2.4) was to be cleaved at the

olefin functionality by use of ozone to give the trihydroxy-aldehyde (**163**), based upon a method of Gensler *et al*[113]. Thus (**178**) was dissolved in ethyl acetate at -78°C and treated with ozone to give a pale purple liquid. After destroying excess O_3 with dimethyl sulfide, the solution became colourless. However, on evaporation of the solvents and analysis, no discernable products were isolated.

Hence it has been shown that a variety of techniques for removal of the benzylidene group from δ-lactol (**157**) resulted in degradation of the products *in situ*. However, treatment with methanol and acid gives the stable acetal (**164**), which may be manipulated to form further derivatives, such as the acetate and methanesulfonate esters (**167**), (**168**) and (**170**). The benzylidene protected δ-lactone (**156**) was also readily deprotected by use of acid and methanol to a stable dihydroxy lactone (**165**). However, repeated attempts to form the aldehydes (**163**) or (**83**) were unsuccessful. Whilst the α,β-unsaturated ketone (**166**) could be obtained reproducibly from (**142**), no further derivatisation from this was possible, nor was isolation of the lactone analogue from (**156**). Interestingly, the treatment of (**157**) with base and either NIS or NBS caused oxidation of the lactol to the lactone (**156**), the proportion of lactone being greater with the stronger oxidant NIS. Lysis of the alkene functionality of (**178**) (section 2.4) by ozonolysis returned no identifiable components. The cumulative information suggests that (**163**) is too unstable to be isolated, possibly degrading by a retro-aldol mechanism, whilst (**83**) was never isolated due to the immediate precursors not being isolated.

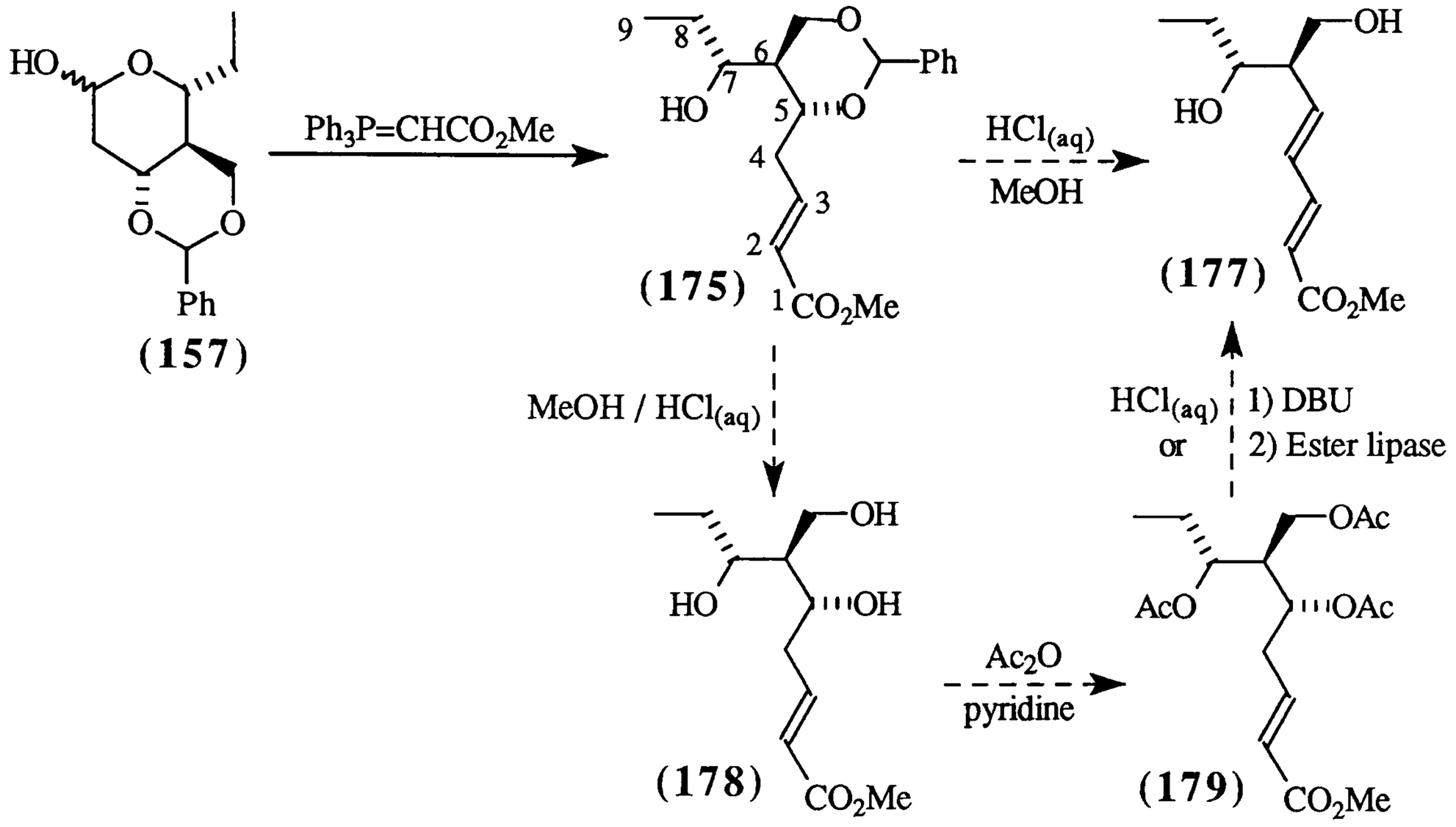
2.4 Studies Towards the Synthesis of Methyl Mycinoate II (**11**)

One of the aims of the current work was to prepare mycinoic acid II from the bicyclic lactone (**82**) *via* the intermediate lactol (**157**). It has previously been shown[75] that a Wittig chain extension of the lactol (**157**) gave the unsaturated ester (**175**). Protection of the secondary alcohol as the SEM ether proceeded smoothly but repeated attempts to remove the benzylidene group (*e.g.*, with $FeCl_3$ on silica; Dowex in MeOH or catalytic hydrogenation) and eliminate to the dienoate (**177**) had failed; the primary alcohol of (**177**) was to have been deoxygenated to yield (**11**).



Scheme 2.439: Previous studies towards the synthesis of methyl mycinoate II[75].

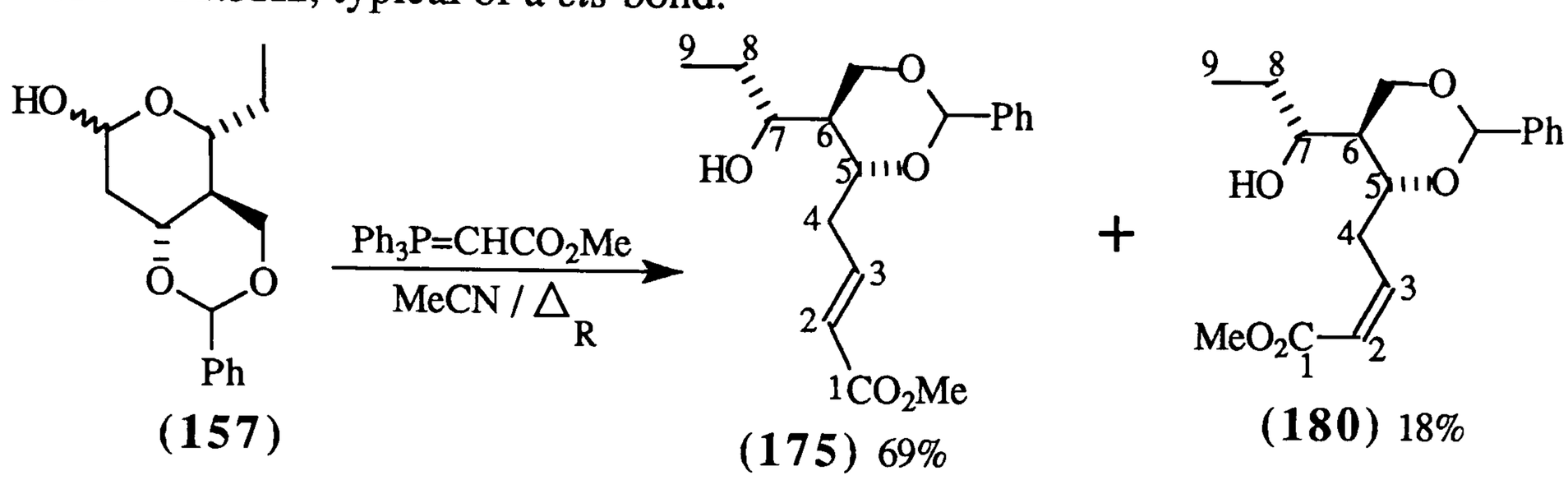
Hence a different approach was required to remove the benzylidene group and to effect the elimination reaction to give the α,β–γ,δ-diunsaturated ester **(177)**.



Scheme 2.40: Proposed approach to mycinoic acid II.

It was proposed to use **(157)** as the substrate for the 2 carbon homologation and then to eliminate the benzylidene group from **(175)** to give the dienoate **(177)** (Scheme 2.40). An alternative approach was to activate the alcohols in the triol **(178)** as good leaving groups (*e.g.*, acetates) and then to selectively eliminate only the 5-hydroxy derivative. One obvious potential flaw with this approach is the need to control the reaction conditions such that all three leaving groups are not concomitantly eliminated. A preferred approach would be to selectively activate the 5-alcohol.

Treatment of δ-lactol **(157)** with methyl (triphenylphosphoranylidene)acetate gave the desired *trans*-α,β-unsaturated ester **(175)** as the major product (69%) which was readily separated from the *cis* isomer **(180)** by column chromatography (Scheme 2.41). The *E*-isomer **(175)** was identified by a characteristic *trans* olefin coupling between 2-H and 3-H of J = 16Hz; the *Z*-isomer **(180)** gave a coupling between 2-H and 3-H of J = 11.5Hz, typical of a *cis* bond.

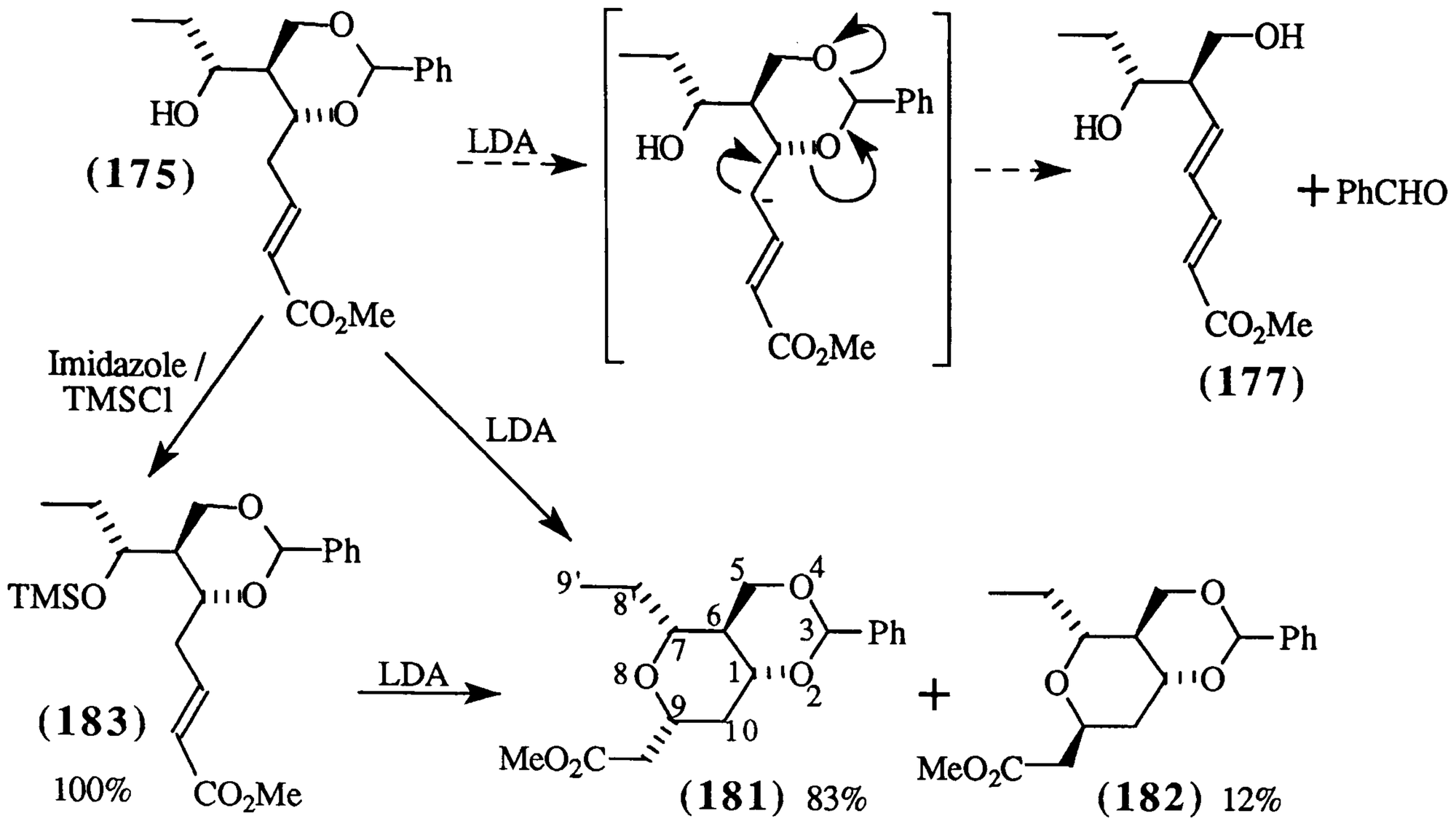


Scheme 2.41: Wittig chain extension of lactol **(157)**.

The *E*-benzylidene protected ester **(175)** was reacted with LDA in an attempt to induce elimination, derived from a technique of Holmes *et al.*[114], by the mechanism shown in Scheme 2.43.

However under the reaction conditions the preferred pathway involved deprotonation of the 7-alcohol followed by a 6-*exo*-trig cyclisation of the resultant alkoxide on C-3 giving the cyclic ethers **(181)** and **(182)**. The two diastereomers were identified by the differences in the ^{1}H NMR spectra of the 10β-H: **(181)** gave ddd of *J*=12, 4.5 and 2Hz at 2.03ppm, which are due to a *geminal* coupling, and two *equatorial - axial* couplings to 1β-H and 9β-H protons; the minor product **(182)** also had a peak split to ddd, at 2.06ppm (*J*=13, 6 and 1.5Hz), the *J* values being typical of a *geminal*, an *equatorial - axial* (10β-H to 1β-H protons) and an *equatorial - equatorial* (10β-H to 9α-H protons) coupling respectively.

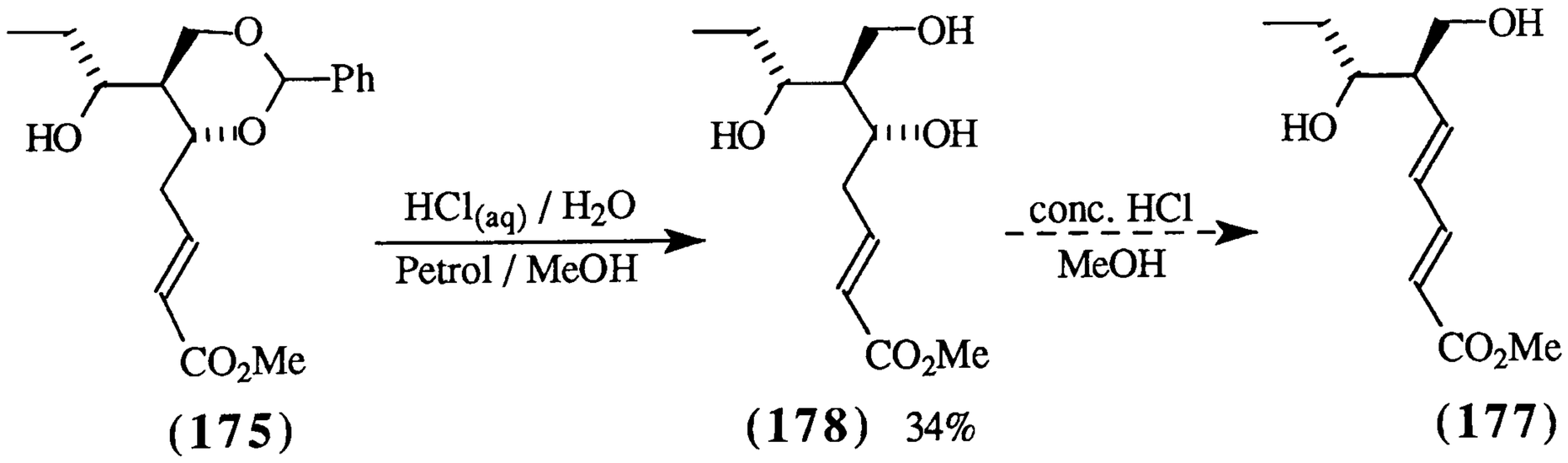
The 7-alcohol in **(175)** was then protected with a TMS group, and the reaction with LDA was repeated upon the silyl alcohol **(183)**. However, whilst TLC, ^{1}H NMR spectra and MS analysis indicated the alcohol had been protected as a silyl ether and **(183)** was stable for over 24 hours at room temperature in air, the products again were the unwanted cyclic ethers.



Scheme 2.42: Proposed and actual results of treating the *E*-ester **(175)** with LDA.

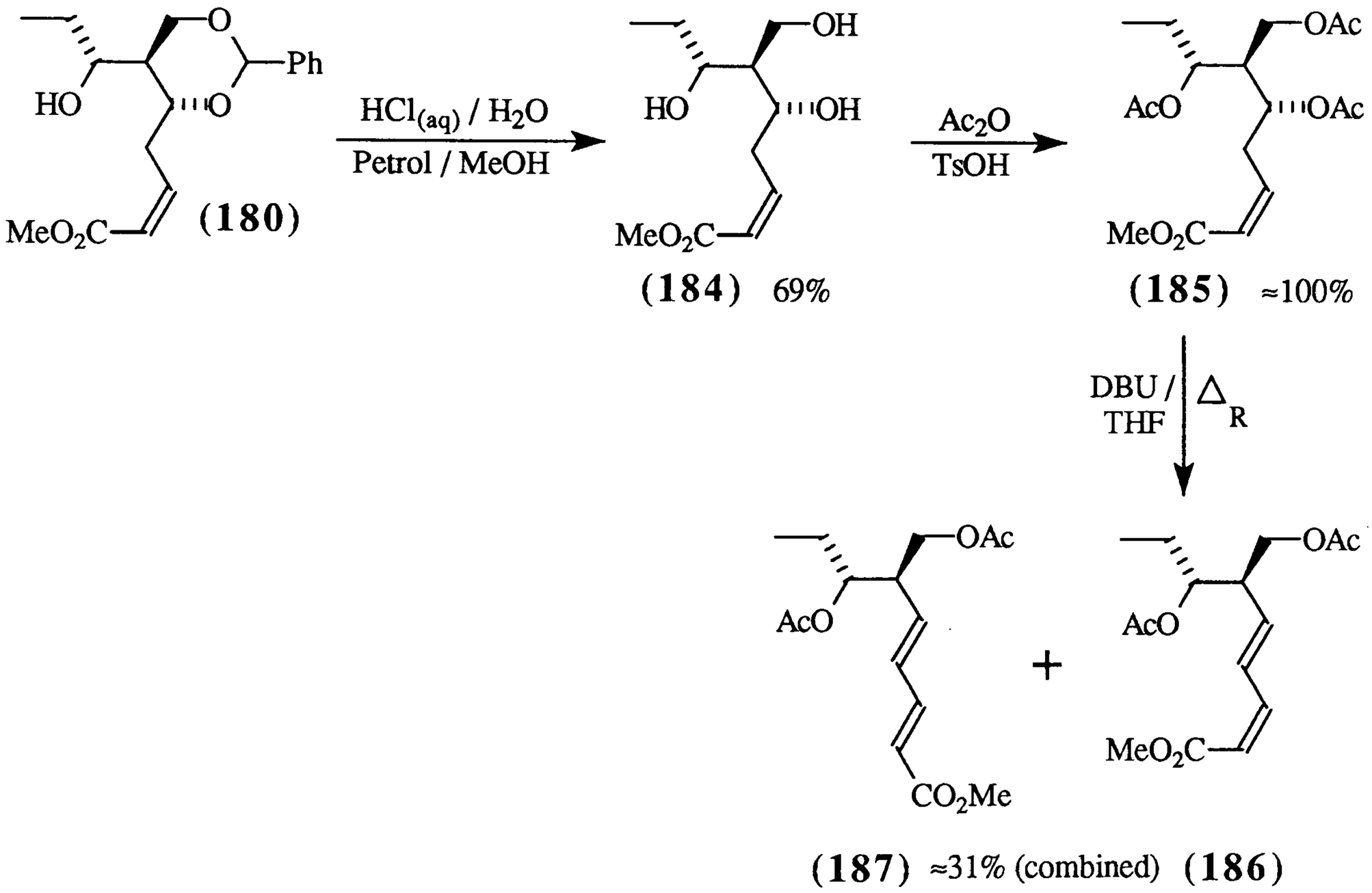
Removal of the benzylidene protecting group by treatment of **(175)** with methanol / petrol / $HCl_{(aq)}$ gave the triol **(178)** in 34% yield. When the reaction was repeated on the *Z*-isomer **(180)**, the corresponding triol **(184)** was isolated in 69% yield.

Stirring *E*-triol **(178)** in MeOH with 10M aqueous hydrochloric acid at either room temperature or 39°C returned starting material quantitatively; heating **(178)** in methanol with Dowex to reflux returned starting material in only 20% yield, but the desired elimination product **(177)** was not characterised by ^{1}H or ^{13}C NMR spectroscopy, although both **(178)** ($M^+ = 232$) and **(177)** ($M^+ = 214$) were tentatively identified by low and high resolution MS.



Scheme 2.43: Acid treatment of the *E*-ester **(119)**.

A different elimination route (scheme 2.44) was conceived using the *Z*-triacetate **(185)**, (no more *E* isomer **(178)** remained to be reacted with the anhydride) which may isomerise during elimination to the *E*-α,β–*E*–γ,δ-diunsaturated ester **(187)**. Use of dilute aqueous hydrochloric acid and MeOH upon **(185)** returned starting material quantitatively; however heating with DBU in THF gave products that were apparently an impure mixture of diacetates *Z*-((**186**))and *E*-((**187**)) in a combined yield of approximately 31%. The ^{1}H NMR spectra of the mixture is given on page 62; the signals at 6.28ppm (dd, *J*=15.5 and 11Hz) and 6.00ppm (dd, *J*=15.5 and 9.5Hz) are characteristic of *trans* olefin bonds conjugated to a carbonyl functionality, suggesting some conversion of **(185)** to the *E*-α,β–*E*–γ,δ-diunsaturated ester **(187)**, whilst those peaks at 5.74ppm (dd, *J*=10.5 and 7Hz) show coupling typical of the *cis* alkene in **(186)**.



Scheme 2.44: Formation and derivatisation of Z-triol.

Due to a lack of further time, no more research into the synthesis towards mycinoic acid II was undertaken.

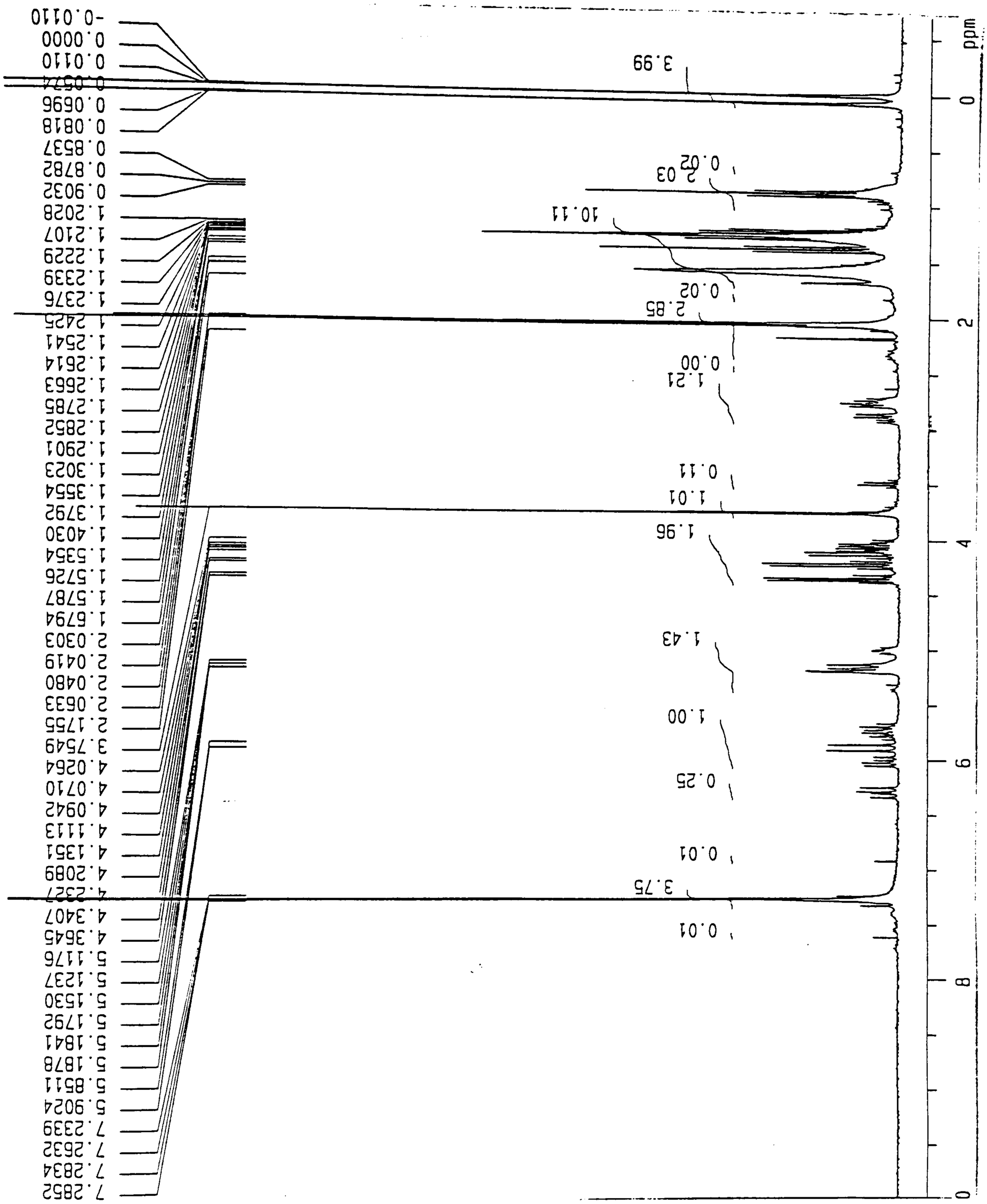
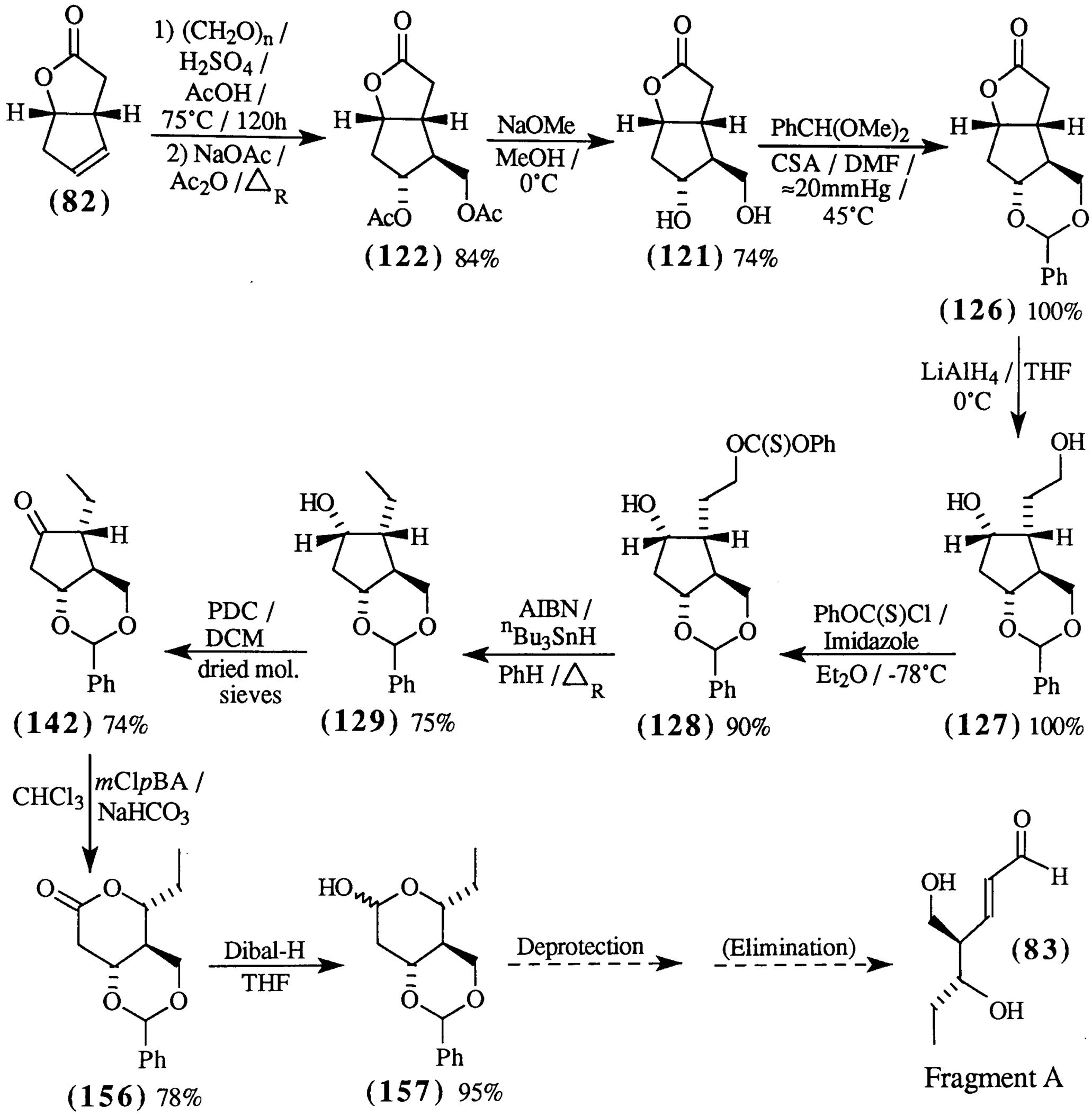


Figure 2.4: ^{1}H NMR spectra (300MHz) of mixture of triesters *Z*-((**186**))and *E*-((**187**)).

2.5 Conclusions and Future Research

The summary of the most successful route to the δ-lactol (**157**) is shown in scheme 2.45, giving (**157**) in 23% yield from lactone (**82**) in 10 steps.



Scheme 2.45: Route and intermediates to fragment A of mycinolide III.

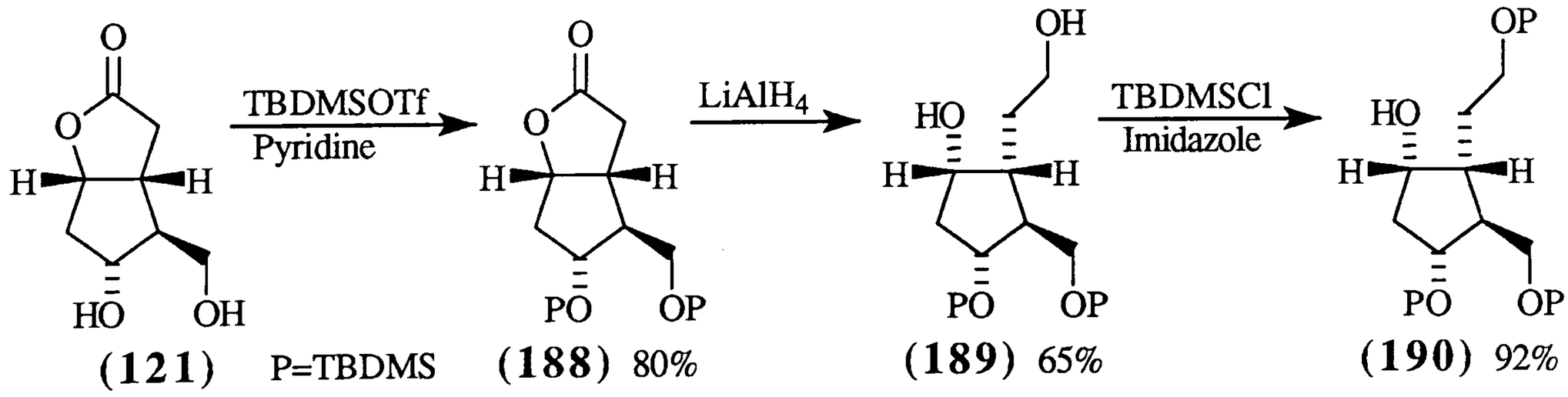
Whilst the synthesis of the target mycinamicin aglycone or mycinoic acids was not acheived, several novel intermediates towards mycinoic acid II were developed, and the addition to (**82**) of a 4α-methyl group gave (**106**), a precursor in the development of fragment B (**83**), in high yield. Many methods of deoxygenation towards an ethyl functionality at C(16) to C(17) of (**39**) were extensively investigated, concluding that the problems with the synthesis of intermediate (**129**) were problably due to the lack of

stability of the primary radical. The problems encountered in the proposed eliminations from a variety of precursors to the target conjugated unsaturated carbonyl compound **(83)** proved insurmountable.

The two main areas requiring further research are the effective synthesis of the ethyl functionality for the C(16)-C(17) portion of the mycinolide and the successful formation of fragment A and by closely related studies, a route to mycinoic acid I.

Further work to obtain the ethyl group of the mycinolide may be undertaken with modified xanthate derivatives, as whilst carbon disulfide is toxic, the development of activated thionoformates, *e.g.*, PhSC(S)OTf, may enable coupling of the primary alcohol of **(127)** to an aromatic xanthate. The reaction between phosgene and selenol gives PhSeC(O)Cl[115], which may be reacted with diol **(127)** to form a selenocarbonate; treatment of the selenocarbonate with a radical hydride should give **(129)**.

Whist previous work on silyl derivatives of diol **(121)** by Robinson[75] had been unsuccessful, with migration of the silicon groups between hydroxyl funcionalities, work recently in the group by Al-Mutairi has enabled stable protection of the alcohol groups of **(121)**[116]. Subsequent opening of the lactone group to a protected diol **(189)** and protection of the ethanolic alcohol in high yield enabled manipulation of the ring hydroxyl. This may allow the deoxygenation step of the ethanolic hydroxyl to give the desired C(16) to C(17) ethyl functionality of mycinolide III **(39)** to be undertaken at a later stage.

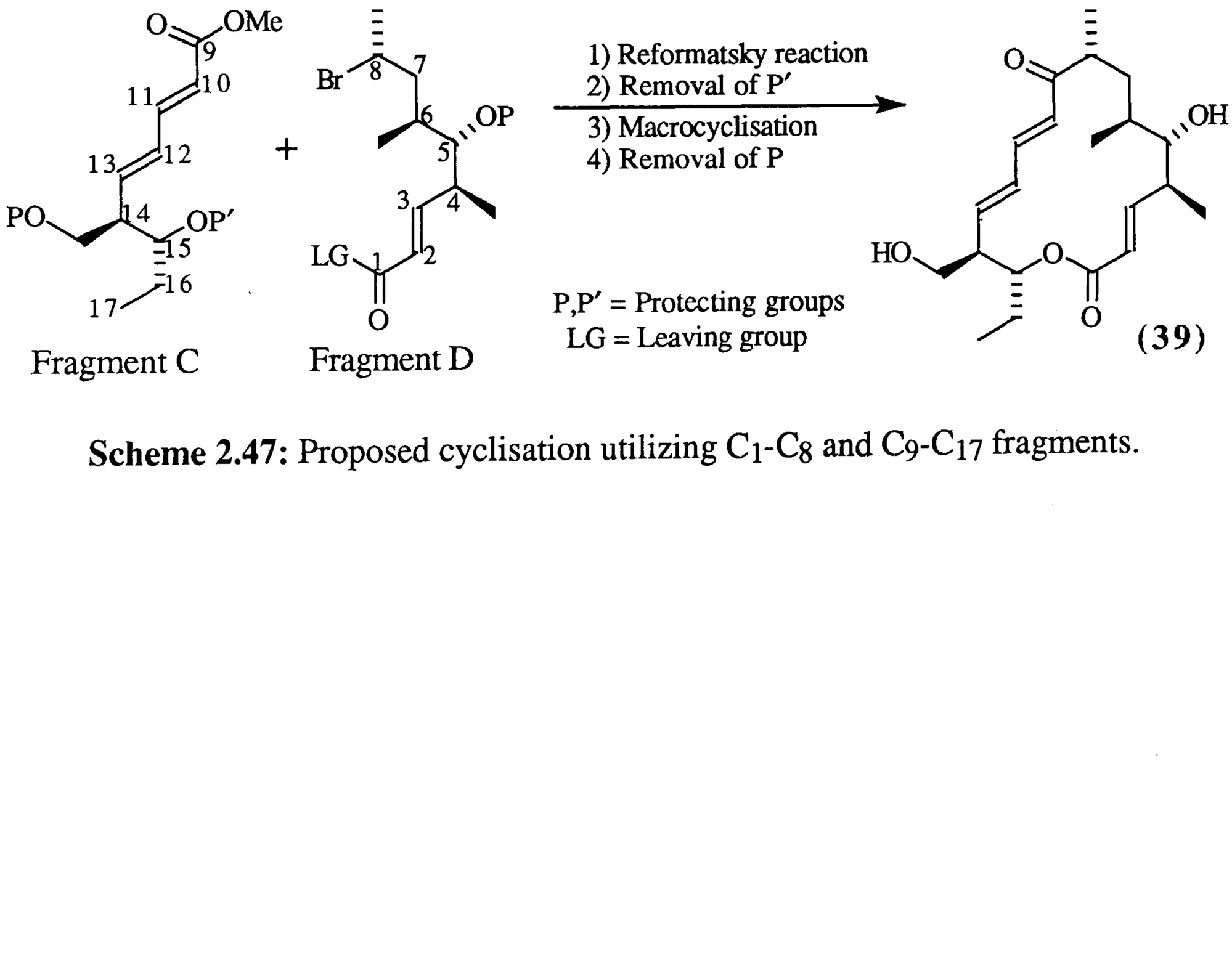


Scheme 2.46: Al-Mutairi route silyl protected polyhydroxyl **(190)**[116].

None of the elimination techniques to date has succeeded in producing the trihydroxy precursor of fragment A **(163)** or fragment A itself **(83)**. Although an

ozonolysis upon the α,β-unsaturated ester (**178**) had failed, the triacetate derivative (**185**) (with the hydroxyl groups protected) might give a stable aldehyde functionality upon treatment with ozone, which should eliminate with ease in syntheses towards fragment A (**83**) and methyl myinoate I (**10**). Alternatively, the proposed and attempted halide introduction into the lactol (**157**) (schemes 2.37 and 2.38) may be modified by use of the δ-lactone (**156**) as the substrate for addition of the haldide α to the carbonyl, which might be reduced to a lactol with Dibal-H and eliminated to the unsaturated aldehyde (**83**) with zinc and acetic acid, as proposed previously.

The research towards the synthesis of the methyl ester of mycinoic acid II (**11**) might be adapted to allow the formation of fragment C, the 14-hydroxymethyl analogue of (**11**). This might be utilized, as proposed in scheme 2.48, as an alternative pathway to the aglycone (**39**).



Fragment C Fragment D

Scheme 2.47: Proposed cyclisation utilizing C_1-C_8 and C_9-C_{17} fragments.

PARTIAL SYNTHESIS OF MYCINOLIDE III AND THE MYCINOIC ACIDS

CHAPTER THREE:

Experimental and References

3 Experimental and References

3.1 General Experimental Details

Glassware for anhydrous reactions was dried under a positive pressure of nitrogen whilst flame-dried with a Bunsen burner. Air or moisture sensitive reagents were added *via* a dropping funnel, Gooch tube, spatula under a fast stream of nitrogen, or injected through Suba-Seals®. Syringes, needles and crushed anhydrous 0.4nm diameter molecular sieves were kept in a drying oven at 142-144°C before use.

Acetonitrile, benzene, DCM, diisopropylethylamine, DMF, ether, THF, toluene, and triethylamine were used in an anhydrous form unless noted otherwise. Ether and THF were redistilled from sodium benzophenone ketyl; benzene and toluene from sodium metal; anhydrous DMF was purchased as such; the remainder were redistilled from calcium hydride. When required, anhydrous methanol was redistilled from magnesium methoxide and iodine; anhydrous acetone was redistilled from magnesium sulfate. Petrol was redistilled before use. When purification was required, other reagents were treated according to the methods of Perrin and Armerego[117].

The crude product of the reaction was obtained by adding aqueous sodium hydrogen carbonate until *p*H ≈8.5 was attained, the organic and aqueous portions separated, and the aqueous layer repeatedly extracted with ethyl acetate. The combined organic liquors were washed with brine, separated from the brine, dried with anhydrous magnesium sulfate, filtered and the solvent evaporated *in vacuo* to give crude product.

All TLC were run on Merck silica gel $60F_{254}$ on aluminium sheets 0.2mm thick in a closed chamber with a saturated atmosphere. Visualisation of TLC plates was by either UV light at 254nm, or an aqueous dip of 5%v/v sulfuric acid, 5%w/v ammonium molybdate tetrahydrate and 0.2%w/v Ceric sulfate, which when heated showed the sample as deep blue colour upon a pale blue background. Dithiane (**10**) was observable with aqueous $KMnO_4$, giving white marks upon a purple background.

Solvents were removed on a rotary evaporator (a Buchi® 461) under water vacuum pressure (≈12mmHg); substances of a higher boiling point were removed under a vacuum of the range 0.1-2mmHg, using a Javac® double stage JDX120 pump.

Flash chromatography columns were made with Fluka 0.035-0.070mm diameter particles. Samples were pre-dried onto silica, then loaded onto the column wetted with petrol, and by eluting with ethyl acetate, petrol and/or methanol, the compounds were isolated, according to a method based on the work of Still and co-workers[118].

Bulb-to-bulb distillation was performed using a Kugelröhr distillation apparatus.

Melting points quoted are uncorrected. These were measured on an Electrothermal® IA6301, of the range 0-360°C.

IR spectra were run on a double beam continuous wave Perkin Elmer® 881 or a Perkin Elmer 1600 FTIR spectrophotometer, the latter marked "*". Solid samples were suspended within KBr discs made in a Specac® 15.011 press at 10 tons pressure; liquids were added directly onto desiccated NaCl plates. Values quoted are for bond stretching.

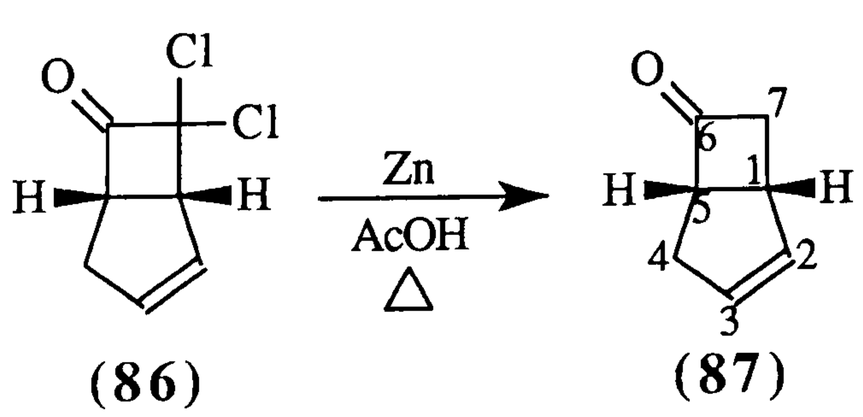
NMR spectra were recorded at room temperature on a Jeol GNM GX270 FT (^{1}H 270MHz, ^{13}C 67.8MHz) spectrometer, except where stated when a GX400 FT (^{1}H 400MHz, ^{13}C 100MHz) or a Lambda (^{1}H 300MHz, ^{13}C 75MHz) was used. Samples were dissolved in deuteriochloroform with tetramethylsilane as the internal standard.

Mass spectra were determined on either a double focussing VG 3D8/RS2MS9 Micromas® (marked "*"), or with a Fisons Autospec®. Samples were submitted in $CHCl_3$ and/or $CDCl_3$, unless otherwise stated. The samples were run *via* EI, unless denoted "CI" or "FAB". High resolution samples were obtained from the Autospec®.

Any deviations from these procedures are noted in the experimental.

3.2 Experimental

(±)-*cis*-Bicyclo[3.2.0]hept-2-en-6-one (87)

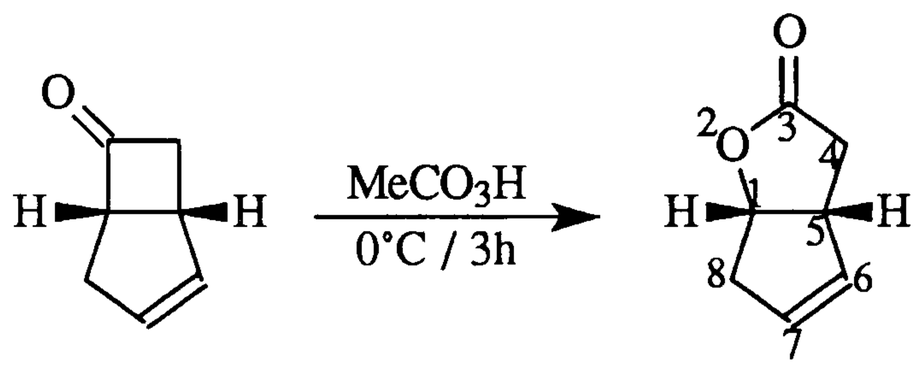


(86) **(87)**

Acetic acid (30ml) was added to zinc powder (54g, 0.83mol) at 0°C, immediately prior to addition of (±)*cis*-7,7-dichlorobicyclo[3.2.0]hept-2-en-6-one **(86)** (30g, 0.17mol) over 1.5h. The reaction mixture was heated to 70°C for 2h. On cooling,

the suspension was filtered. Potassium carbonate powder and saturated aqueous solution were added to the filtrate until the solution became *p*H 5. The organic layer was separated and the aqueous layer extracted with DCM (3 x 100ml portions). The combined organic layers were washed with distilled water, brine, dried over anhydrous magnesium sulfate and filtered. The solvent was evaporated *in vacuo*, and the product distilled at 16mmHg at 60°C to give the bicyclic ketone **(87)**, a clear moderately free - flowing liquid, (18.1g, 1.68mol, 99%)[119]; R_f = 0.54 (ethyl acetate : petrol, 20:80); δ_H 2.49 (1H, m, 7β-H), 2.69 (2H, m, 4-H_2), 3.32 (1H, m, 7α-H), 3.48 (1H, m, 1-H), 3.87 (1H, br m, 5-H) and 5.82 (2H, m, 2-H, 3-H); *m/z** 108 (M^+, 4%), 95 (49), 91 (24), 83 (43), 82 (28), 81 (37), 79 (44) and 47 (100).

(±)-*cis*-2-Oxabicyclo[3.3.0]oct-6-en-3-one (82)

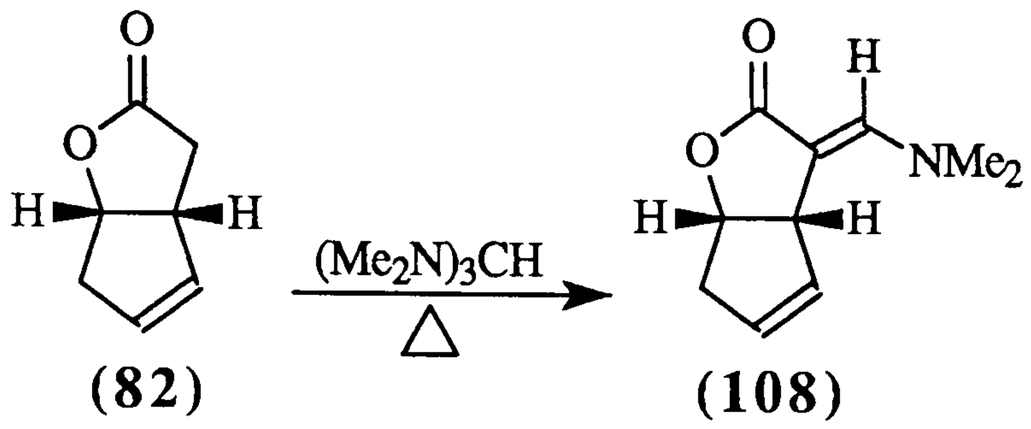


(87) **(82)**

Glacial acetic acid (70ml) and the ketone **(87)** (27.0g, 0.25mol) were stirred at 0°C; a mixture of 30% w/v hydrogen peroxide (30ml, 0.29mol peroxide) and acetic acid (30 ml) at 0°C was added over 3h. Maintaining a temperature of 0°C, excess saturated aqueous sodium sulfite solution was added, then potassium carbonate (aqueous and solid) was added to *p*H 5. Extraction of the aqueous layer with ethyl acetate (4 x 100ml), followed by washing the combined ethyl acetate fractions with brine and saturated potassium carbonate, separation from the aqueous layer, drying with anhydrous magnesium sulfate, filtration and the solvent evaporation *in vacuo* yielded the lactone **(82)** (31.03g, ≈100% yield), a clear, colourless slightly viscous liquid which had a pleasant and sweet odour[119]; R_f = 0.55 (ethyl acetate : petrol, 50:50); υ_{max}/cm^{-1} 1620 (C=C), 1770 (C=O) and 3059 (C-H, olefinic); δ_H 2.45 (1H, dd, *J* 18 and 1.5, 4β-H), 2.72 (2H, m, 8-H_2), 2.79 (1H, dd, *J* 18 and 9.5, 4α-H), 3.53 (1H,

m, 5-H), 5.14 (1H, m, 1-H), 5.60 (1H, m, 7-H) and 5.80 (1H, m, 6-H); *m/z* 124 (M^+, 53%), 96 (60), 95 (87), 81 (48), 79 (43), 68 (30), 67 (100) and 66 (26).

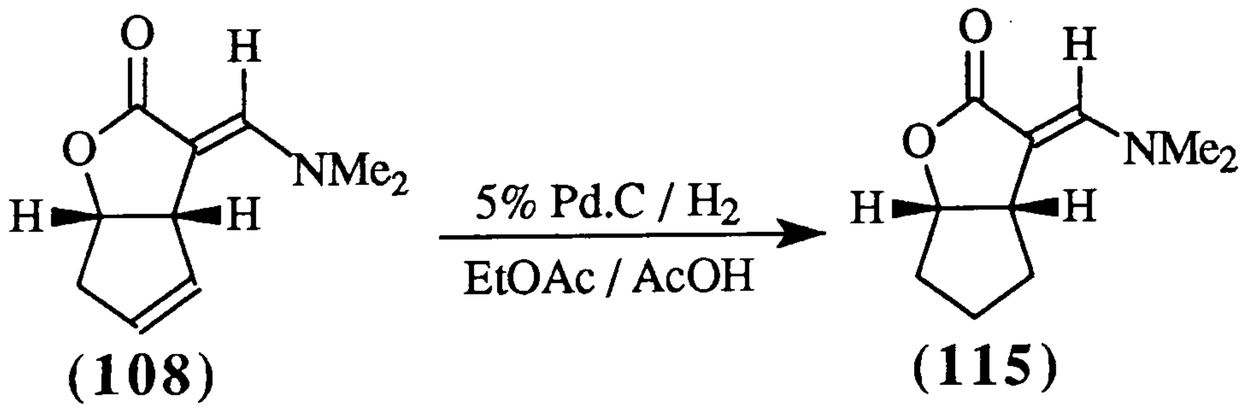
(±)-*cis*-4-(Dimethylaminomethylene)-2-oxabicyclo[3.3.0]-oct-6-en-3-one (108)



(82) **(108)**

Lactone **(82)** (0.124g, 1.0mmol) and tris(dimethylamino)methane (0.2ml, 1.16mmol) were heated at 75°C for 5h with stirring under nitrogen. On cooling, the reaction mixture was purified by column chromatography eluting with ethyl acetate : petrol (60:40), yielding the vinylagous carbamate **(108)**, a yellow solid in yellow liquor (which could not be recrystallised), (0.88g, 4.92mmol, 49%)[65]; R_f = 0.53 (ethyl acetate); δ_H 2.73 (2H, m, 8-H_2), 3.09 (6H, s, 2 x CH_3), 4.24 (1H, dd, *J* 5.5 and 1.5, 5-H), 4.99 (1H, m, 1-H), 5.59 (1H, m, 7-H), 5.71 (1H, m, 6-H) and 7.12 (1H, d, *J* 1.5, 4-C<u>H</u>-N$(CH_3)_2$); *m/z* * 179 (M^+, 62%), 134 (29), 115 (37), 103 (74), 91 (93), 75 (65) and 43 (100).

(±)-*cis*-4-(Dimethylaminomethylene)-2-oxabicyclo[3.3.0]octan-3-one (115)

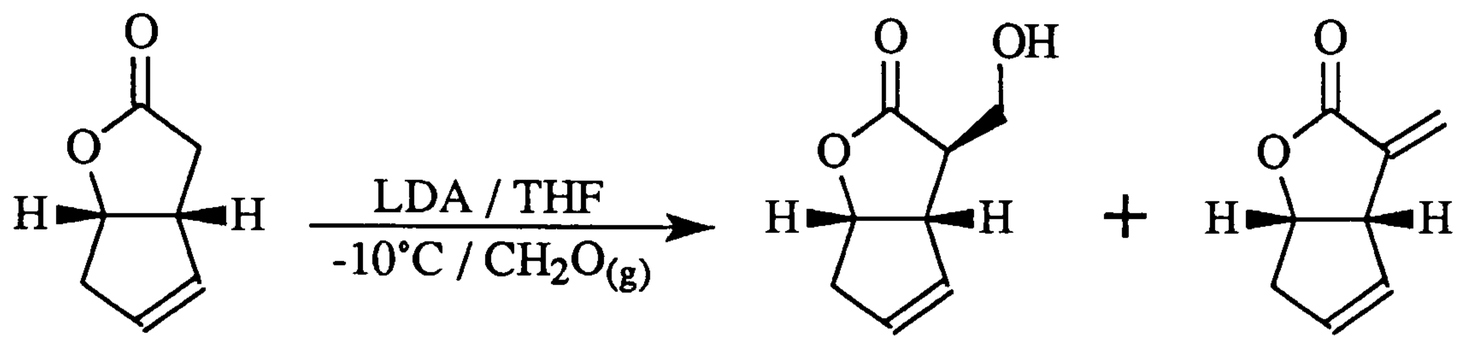


(108) **(115)**

The enamine **(108)** (179mg, 1mmol) was dissolved in ethyl acetate (15ml) and acetic acid (1ml), and 5% palladium on Degussa® carbon (50mg, 0.0235mmol) added. The reaction mixture was stirred under an atmosphere of hydrogen gas. 23.5ml (0.979mmol) of hydrogen was absorbed. The suspension was filtered and evaporated *in vacuo*, to give the crude product (65mg). The solid was purified by column chromatography using ethyl acetate : petrol (50:50), to give the viscous deep brown

vinylagous carbamate **(115)**, (167mg, 0.92mmol, 92%); R_f = 0.38 (ethyl acetate : petrol, 65:35); (Found: M^+, 181.1103. $C_{10}H_{15}NO_2$ requires M, 181.1103); υ_{max}/cm^{-1} 1627 (C=C), 1719 (N-H) and 1767 (C=O); δ_H 1.71 and 2.01 (6H, m, 6-H_2, 7-H_2, 8-H_2), 3.01 (6H, s, 2 x CH_3), 3.59 (1H, m, 5-H), 4.81 (1H, m, 1-H) and 7.06 (1H, d, *J* 1.5, 4-C<u>H</u>-N$(CH_3)_2$); δ_C 22.91, 34.16 and 36.45 (C-6, 7, 8), 41.56 ($(CH_3)_2$), 41.75 (C-5), 82.16 (C-1), 93.64 (C-4), 146.96 (4-<u>C</u>H-N$(CH_3)_2$) and 176.15 (C-3); *m/z* 181 (M^+, 67%), 166 (5), 152 (44), 138 (15), 136 (20), 124 (8), 111 (13), 97 (37) and 77 (58).

(±)-*cis*-4β-Hydroxymethyl-2-oxabicyclo[3.3.0]oct-6-en-3-one (116) and **(±)-*cis*-4-methylen-2-oxabicyclo[3.3.0]oct-6-en-3-one (109)**



(82) **(116)** **(109)**

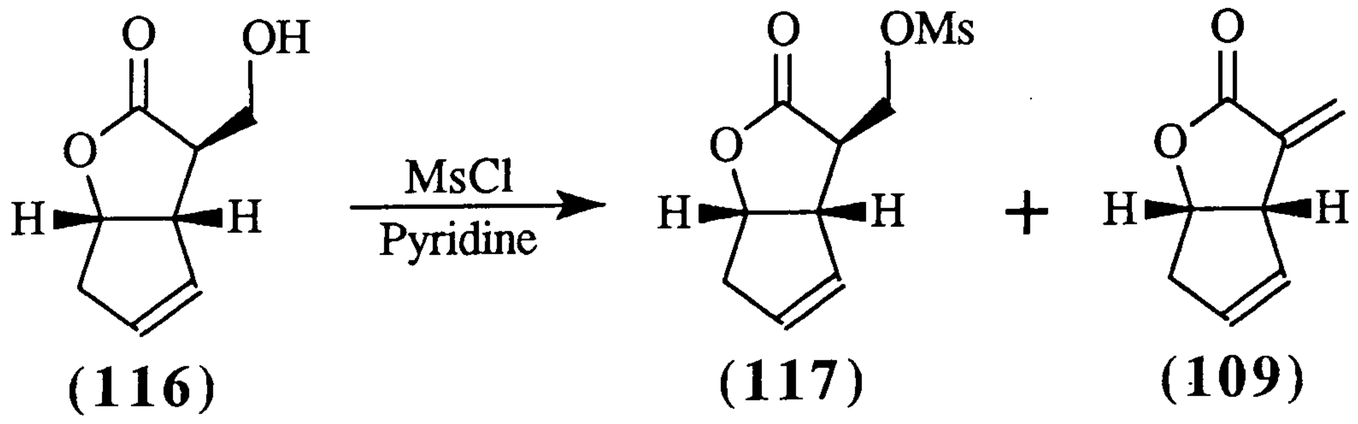
n-Butyl lithium (2.0ml of a 2.5M solution in hexanes, 5mmol) was added to diisopropylamine (0.9ml, 6.4mmol) in THF (50ml) at 0°C, and stirred for 0.5h to prepare a solution of LDA. The temperature was then lowered to -78°C. Lactone **(82)** (507mg, 4.09mmol) was added directly by injection and the temperature was allowed to reach -10°C for 2h to form the enolate.

Dried paraformaldehyde was transferred to a dried 2-neck round bottom flask and nitrogen pumped over the formaldehyde and out through a glass tube of 0.25 inch diameter which had Quickfit® inlet for insertion into the reaction vessel. Nitrogen pressure was increased to the maximum rate into the 2-neck flask, which was lowered into a silicone bath at 140°C (below the evaporation point of formaldehyde), and the glass tube was then inserted into a port of the enolate - containing flask with a pressure outlet fitted to the enolate flask. After approximately 2g of formaldehyde had passed over, the formaldehyde tube was removed, the inlet port was stoppered and the reaction flask left under anhydrous conditions at 0°C for 3h.

The reaction mixture, which appeared as a mixture of several compounds by TLC analysis, was quenched with 1M hydrochloric acid (approximately 20ml), and extracted by the standard method, but for acidic conditions. The crude product was purified by column chromatography, eluting with ethyl acetate : petrol (30:70), to give the colourless liquid dialkene **(109)** (35mg, 0.257mmol, 6%)[120]; R_f = 0.73 (ethyl acetate : petrol, 50:50); δ_H 2.68 (2H, m, 8-H_2), 3.97 (1H, m, 5-H), 5.04 (1H, m, 1-H), 5.47 (1H, m, 7-H), 5.62 (1H, d, *J* 2, 4-C<u>H</u>H), 5.71 (1H, m, 6-H) and 6.12 (1H, d, *J* 2, 4-CH<u>H</u>); *m/z** 136 (M^+, 49%), 108 (61), 107 (68), 79 (100) and 77 (58).

Further elution with ethyl acetate : petrol (60:40) gave the alcohol **(116)** as a clear liquid (406mg, 2.64mmol, 65%); R_f = 0.25 (ethyl acetate : petrol, 50:50); (Found: M^+, 154.0635. $C_8H_{10}O_3$ requires M, 154.0630); υ_{max}/cm^{-1} 1764 (C=O) and 3449 (H-O); δ_H 2.63 (1H, m, 4-H), 2.72 (2H, m, 8-H_2), 3.47 (1H, m, 5-H), 3.88 (1H, dd, *J* 11 and 4.5, 4-C<u>H</u>HOH), 4.02 (1H, dd, *J* 11 and 4.5, 4-CH<u>H</u>OH), 4.87 (1H, br s, 4-CH_2O<u>H</u>), 5.17 (1H, m, 1-H), 5.63 (1H, m, 7-H) and 5.79 (1H, m, 6-H); δ_C 39.43 (C-8), 48.70 (C-5), 49.84 (C-4), 62.54 (4-CH_2OH), 83.27 (C-1), 129.47 (C-7), 131.05 (C-6) and 179.06 (C-3); *m/z* 154 (M^+, 6%), 136 (12), 124 (100), 108 (18), 107 (19) and 106 (22).

(±)-*cis*-4β-Methanesulfonoxymethyl-2-oxabicyclo[3.3.0]oct-6-en-3-one (117) and **(±)-*cis*-4-methylen-2-oxabicyclo[3.3.0]oct-6-en-3-one (109)**



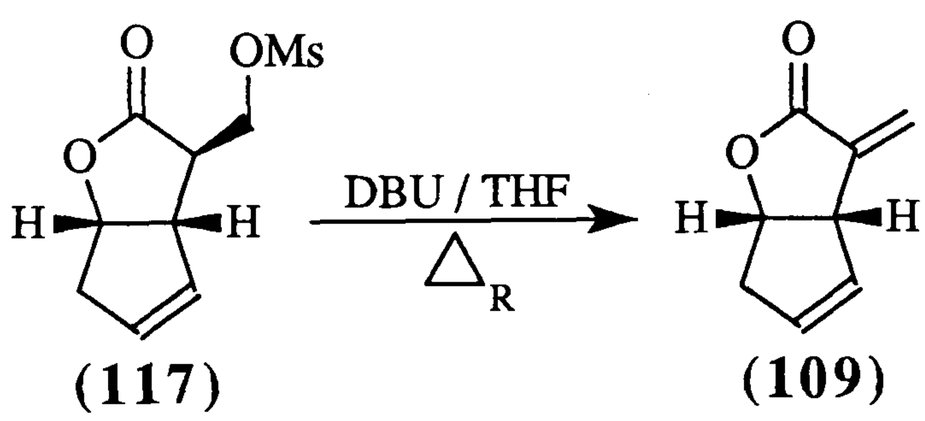
(116) **(117)** **(109)**

The alcohol **(116)** (310mg, 2.5mmol) was dissolved in pyridine (10ml) at room temperature and methanesulfonyl chloride (1.5ml, 19.4mmol) was added with stirring, without allowing the temperature to rise above 18°C. After 2.5h dilute hydrochloric acid (50ml) was added to make the system acidic and extracted as normal. The crude product was purified by column chromatography (using ethyl acetate : petrol, 30:70) to

yield the α, β unsaturated lactone (**109**) (60mg, 0.441mmol, 18%). Spectral data as before.

Further elution using ethyl acetate : petrol (50:50), gave the mesylate (**117**) (270mg, 1.16mmol, 47%) as fluffy white crystals; mp 70-72°C, (from carbon tetrachloride); R_f = 0.50 (ethyl acetate : petrol, 50:50); (Found C, 46.8; H, 5.2; S, 13.5%; $[MH]^+$, 233.0482. $C_9H_{12}O_5S$ requires C, 46.5; H, 5.2; S, 13.8%; MH, 233.0484); υ_{max}/cm^{-1} 1361 and 1170 (S=O) and 1765 (C=O); δ_H 2.75 (2H, m, 8-H_2), 2.81 (1H, td *J* 4 and 2, 4-H), 3.05 (3H, s, CH_3), 3.55 (1H, ap dtd, *J* 6.5, 4 and 2, 5-H), 4.52 (2H, m, 4-C$\underline{H}_2$), 5.19 (1H, m, 1-H), 5.64 (1H, m, 7-H) and 5.84 (1H, m, 6-H); δ_C 37.43 (C-8), 39.34 (C-5), 45.69 (CH_3), 49.30 (C-4), 69.17 (4-$\underline{C}H_2OMs$), 82.89 (C-1), 130.05 (C-7), 130.48 (C-6) and 175.60 (C-3); *m/z* (CI) 233 ($[MH]^+$, 100%), 219 (28), 151 (11), 137 (97), 136 (22), 119 (30), 93 (71) and 91 (46).

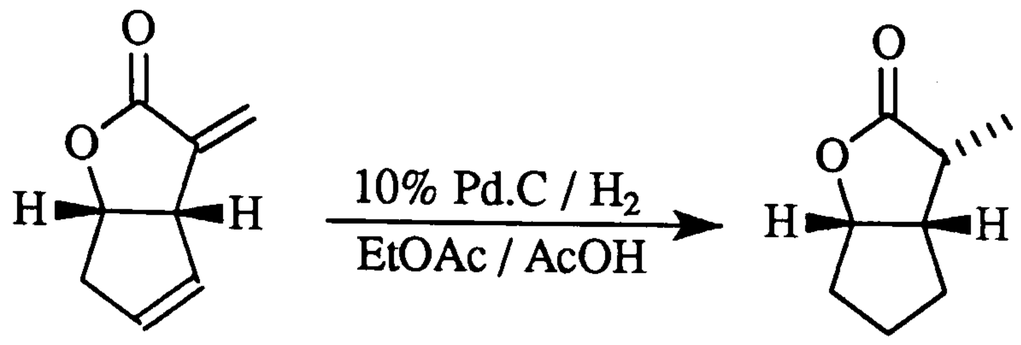
Repeated synthesis of exocyclic alkene (109)



(117) **(109)**

The mesylate (**117**), (116mg, 0.50mmol) was dissolved in THF (30ml) with DBU (0.1ml, 0.67mmol), and heated to reflux for 1.25h. After cooling, the reaction mixture was poured into dilute hydrochloric acid and extracted as for the lactone (**82**). No further purification of the methylene lactone (**109**) was required, (70mg, >100% possible crude yield). Spectral data as previously reported.

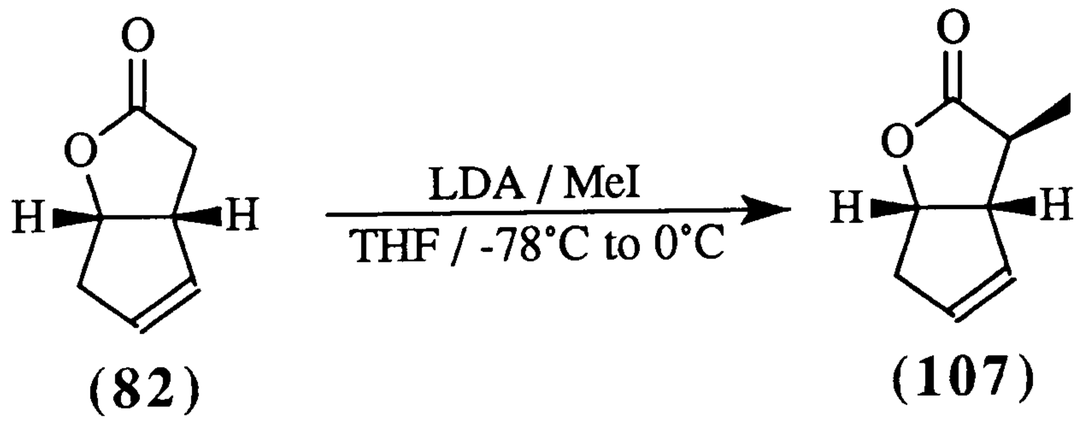
(±)-*cis*-4α-Methyl-2-oxabicyclo[3.3.0]oct-3-one (110)



(109) **(110)**

The exocyclic alkene **(109)** (70mg, 0.51mmol), in ethyl acetate (25ml) and glacial acetic acid (0.5ml), was stirred with 10% palladium on carbon (35mg, 0.0329mmol) under an atmosphere of hydrogen gas for 0.75h, (25.2ml, 1.05mmol H_2 taken up). The suspension was then filtered, the flask rinsed with ethyl acetate : petrol (50:50) (3 x 20ml), and evaporated *in vacuo*. The crude solid was then purified by column chromatography, using ethyl acetate : petrol (20:80), to give the saturated lactone **(110)**, as a gum (55mg, 0.39mmol, 77%); R_f = 0.46 (ethyl acetate : petrol, 30:70); (Found $[MH]^+$, 141.0910. $C_8H_{13}O_2$ requires MH, 141.0916); δ_H 1.21 (3H, d, *J* 7, 4-CH_3), 1.79 (6H, m, 6-H_2, 7-H_2, 8-H_2), 2.79 (1H, m, 5-H), 2.84 (1H, dq, *J* 8.5 and 7, 4-H) and 4.87 (1H, td, *J* 5.5 and 1.5, 1-H); δ_C 11.25 (4-CH_3), 24.27, 25.89 and 32.82 (C-6, C-7, C-8), 38.15 (C-5), 44.11 (C-4), 84.32 (C-1) and 179.66 (C-3); *m/z* (CI) 141 ($[MH]^+$, 52%), 123 (12), 113 (5), 111 (5), 97 (8), 95 (34), 85 (8) and 67 (9).

(±)-*cis*-4β-Methyl-2-oxabicyclo[3.3.0]oct-6-en-3-one (107)

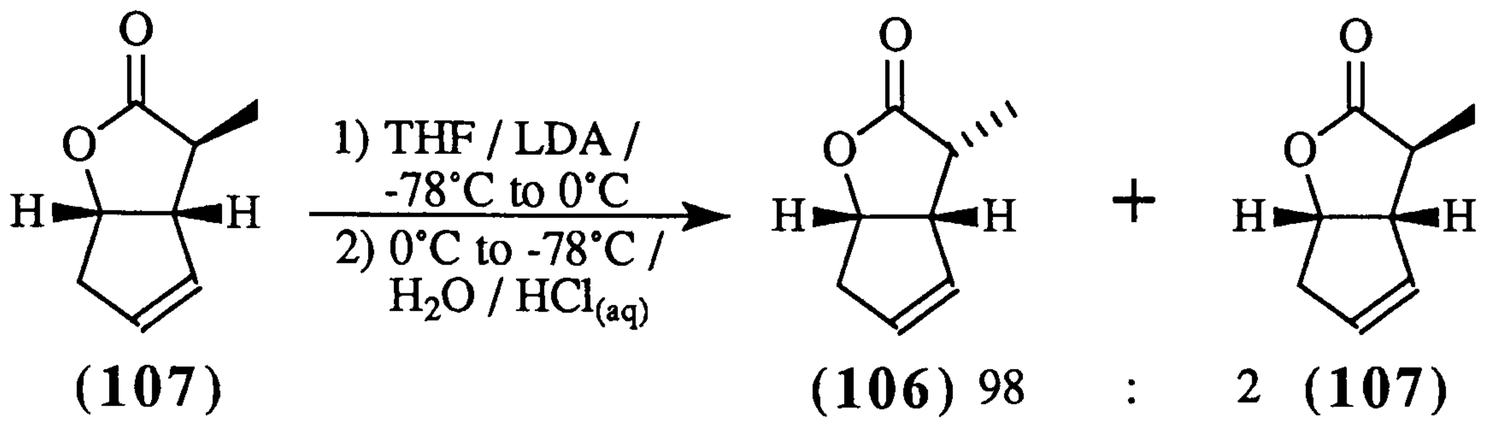


(82) **(107)**

LDA (5mmol) in THF (40ml) was made by the same method as for synthesis of **(116)**. The temperature was then lowered to -78°C. The lactone **(82)** (478mg, 3.85mmol) was added and stirred for 1h, to form the enolate. Methyl iodide (0.3ml, 4.82mmol) was added and the reaction mixture allowed to warm to 0°C over 1.2h. The reaction was quenched with 3M hydrochloric acid (10ml) and extracted as usual. The residue was not purified, as the ^1H NMR and MS spectra of 4β-methyl lactone **(107)**

corresponded with previous data, (530mg, 3.84mmol, 99%)[65]; R_f = 0.56 (ethyl acetate : petrol, 25:75); δ_H 1.38 (3H, d, *J* 7.5, 4-CH_3), 2.56 (1H, qd, *J* 7.5 and 2, 4-H), 2.70 (2H, m, 8-H_2), 3.16 (1H, m, 5-H), 5.16 (1H, m, 1-H), 5.62 (1H, m, 7-H) and 5.78 (1H, m, 6-H); *m/z** 138 (M^+, 14%), 123 (2), 109 (14), 79 (51) and 77 (17).

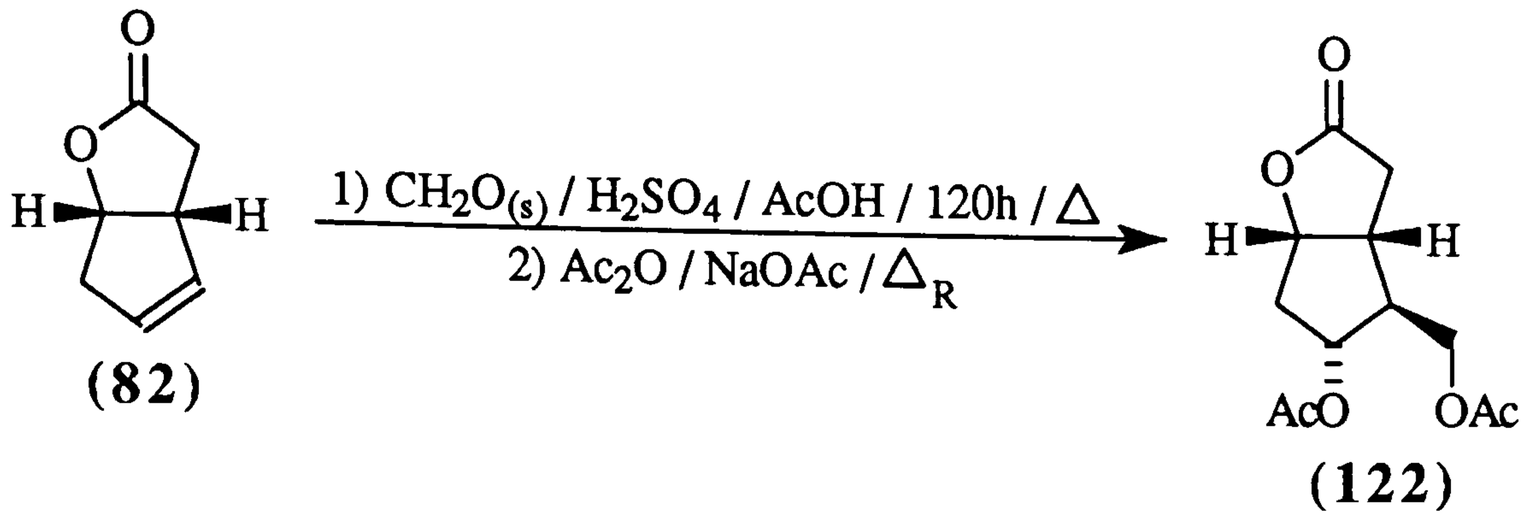
(±)-*cis*-4α-Methyl-2-oxabicyclo[3.3.0]oct-6-en-3-one (106) and **(±)-*cis*-4β-Methyl-2-oxabicyclo[3.3.0]oct-6-en-3-one (107)**



(107) **(106)** 98 : 2 **(107)**

LDA (1.1mmol) was prepared as previously and the temperature was lowered to -78°C. The 4β-methyl lactone **(107)** (138mg, 1mmol) was added and over 1.25h warmed to ≈0°C, recooled to -78°C, and water (0.9ml) was added. After 1.5h the temperature had risen to -50°C, then the reaction mixture was warmed to room temperature and the crude product extracted as for **(107)**; yielding the 4α-methyl lactone, a pale yellow very viscous liquid (0.14g, ≈100% yield). The crude product was purified by column chromatography (using ethyl acetate : petrol ratios, 20:80) to yield as a colourless liquid the 4α-methyl lactone **(106)** (136mg, 0.986mmol, 99%; with 96% de, by comparison of integrals due to methyl protons on the ^1H NMR spectra); R_f = 0.50 (ethyl acetate : petrol, 25:75); (Found: M^+, 138.0676. $C_8H_{10}O_2$ requires M, 138.0681); υ_{max}/cm^{-1} 1764 (C=O) and 3061 (C=C); δ_H 1.27 (3H, d, *J* 7.5, 4-CH_3), 2.71 (2H, m, 8-H_2), 2.85 (1H, dq, *J* 9 and 7.5, 4-H), 3.54 (1H, m, 5-H), 5.02 (1H, m, 1-H), 5.66 (1H, m, 7-H) and 5.83 (1H, m, 6-H); δ_C 11.90 (4-CH_3), 37.28 (C-5), 39.59 (C-8), 50.51 (C-4), 80.52 (C-1), 127.29 (C-7), 130.57 (C-6) and 178.72 (C-3); *m/z* 138 (M^+, 74%), 124 (37), 109 (41), 107 (36), 94 (78), 93 (82), 83 (69) and 79 (100).

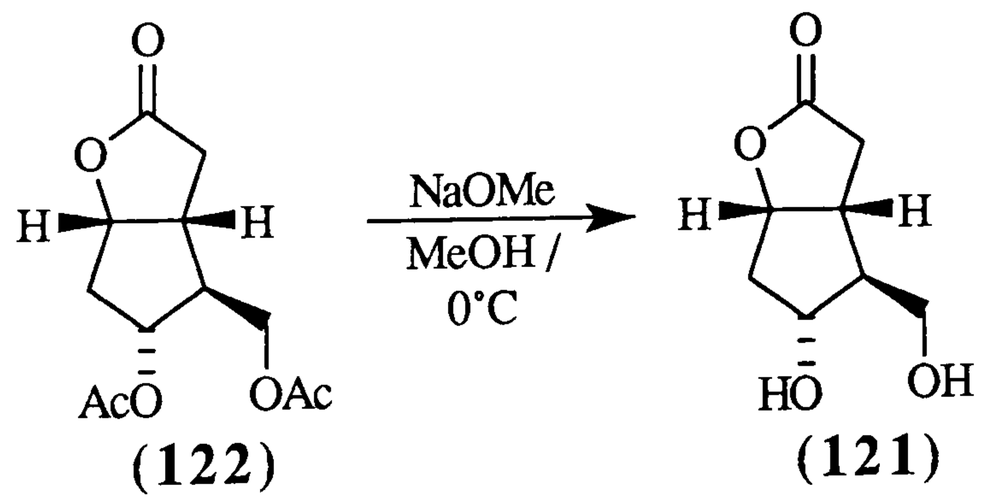
(±)-*cis*-7α-Acetoxy-6β-acetoxymethyl-2-oxabicyclo[3.3.0]octan-3-one (122)



(82) **(122)**

Paraformaldehyde (10.0g, 333mmol) was added to a mixture of concentrated sulfuric acid (2.5ml) and glacial acetic acid (60ml), and heated to reflux for 0.75h, forming a transparent and colourless solution. After cooling to 25°C, the lactone (**82**) (10g, 80.6mmol) in acetic acid (10ml) was added and the reaction heated at 75°C for 5 days. Sodium acetate (10.0g, 122mmol) in Ac_2O (10ml, 106mmol) was added and the solution heated to reflux for 5h. On cooling, the reaction was raised to *p*H 5 with saturated aqueous potassium carbonate and extracted as usual. The dried residue was distilled with bulb-to-bulb apparatus, yielding the diacetate (**122**), a clear and colourless liquid; (17.314g, 67.6mmol, 84%); bp 150-175°C/0.1mmHg[77]; R_f = 0.38 (ethyl acetate : petrol, 65:35); υ_{max}/cm^{-1} 1740 (C=O acetates) and 1775 (C=O lactone); δ_H 1.99 and 2.03 (each 3H, each s, each O_2CCH_3), 2.48 (dd, *J* 18 and 2, 4β-H), 2.85 (dd, *J* 18 and 11, 4α-H), 2.70, 2.31 and 2.16 (4H, m, 6-H, 5-H, 8-H_2), 3.98 (2H, m, 6-CH_2), 4.98 (2H, m, 1-H, 7-H); *m/z* (CI) 257 ($[MH]^+$, 2%), 243 (1), 227 (2), 215 (20), 198 (16), 197 (100), 183 (17) and 137 (51).

(±)-*cis*-7α-Hydroxy-6β-hydroxymethyl-2-oxabicyclo[3.3.0]octan-3-one (121)

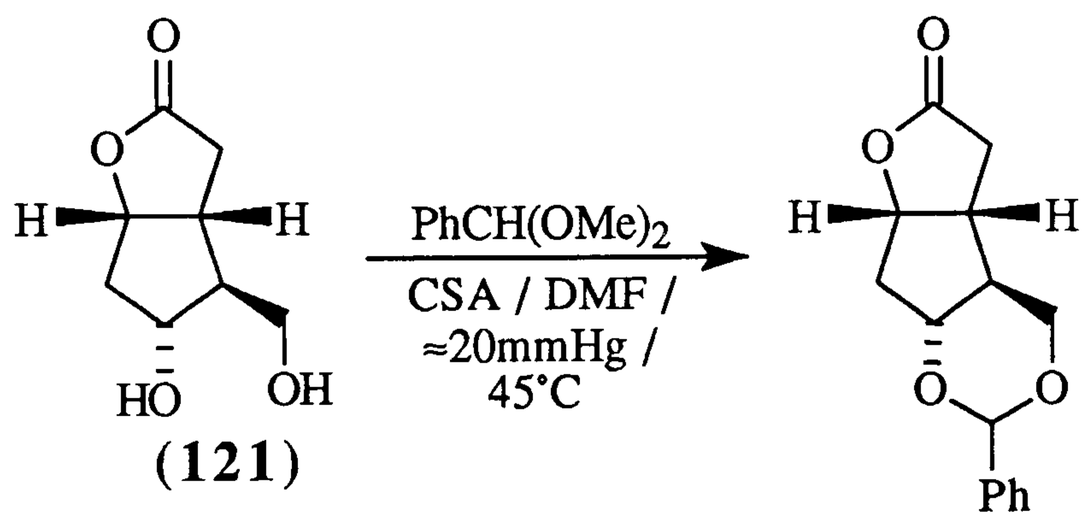


(122) **(121)**

The diacetate (**122**) (5.85g, 23mmol) in dry distilled MeOH (10ml) was added to a stirred solution of sodium methoxide at 0°C, (prepared from sodium (0.38g, 17mmol) and dry distilled MeOH (20ml) in an ice bath). The reaction was stirred for

2h, warming to room temperature; glacial acetic acid was then added until *p*H 4 was obtained and the solvent evaporated *in vacuo*. The crude product was purified by column chromatography eluting with MeOH : ethyl acetate (10:90), yielding the diol **(121)**, a very viscous colourless liquid, (2.86g, 16.6mmol, 74%)[121]; R_f = 0.28 (methanol : ethyl acetate, 10:90); υ_{max}/cm^{-1} 1762 (C=O) and 3400 (O-H); δ_H ($(CD_3)_2CO$) 1.94 (2H, m, 8-H_2), 2.32 (1H, m, 5-H), 2.47 (1H, d, *J* 15, 4β-H), 2.76 (1H, m, 6-H), 2.79 (1H, dd, *J* 18.5 and 10.5, 4α-H), 3.48 (2H, m, 6-CH_2), 4.09 (1H, m, 7-H), 4.93 (1H, td, *J* 7 and 2.5, 1-H); *m/z* ($(CD_3)_2CO$, CI) 173 ($[MH]^+$, 77%), 155 (44), 138 (16), 137 (100), 125 (63), 109 (25) and 91 (29).

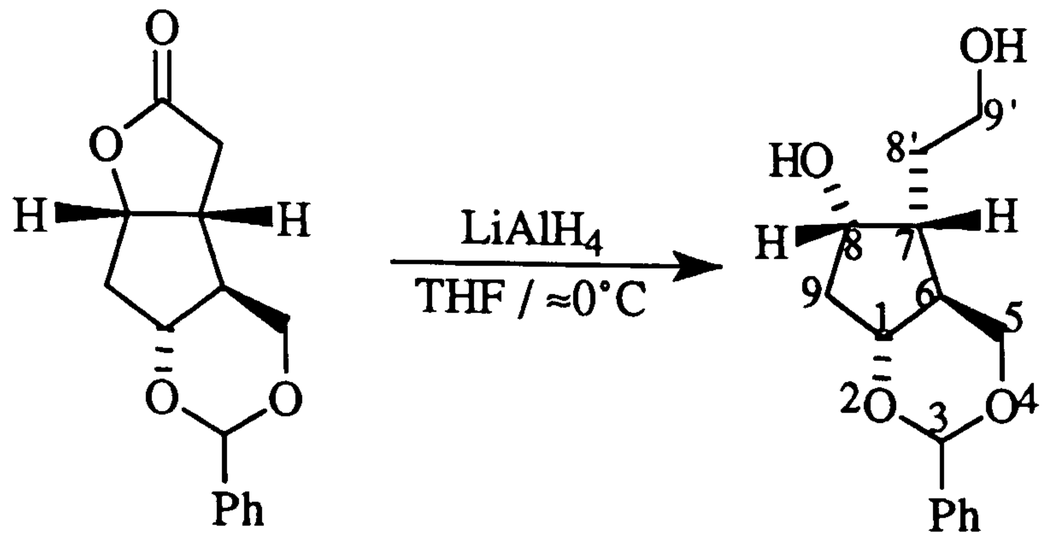
Synthesis of the benzylidene derivative of (±)-*cis*-7α-hydroxy-6β-hydroxymethyl-2-oxabicyclo[3.3.0]octan-3-one (126)



(121) **(126)**

To a solution of the diol **(121)**, (121mg, 0.703mmol) in dry DMF (20ml) was added CSA (20mg, 0.0862mmol) and benzaldehyde dimethyl acetal (0.25ml, 1.67mmol). The reaction mixture was heated at 45°C for 1.75h at 20mmHg pressure with an air cooled reflux condenser. The reaction mixture was extracted by the standard procedure, followed by drying at 0.2mmHg for 1.5h at ≈40°C (removing the DMF) to give the acetal protected lactone **(126)** as a cream coloured solid, (190mg, >100%), which corresponded with previous data reported; R_f = 0.21 (ethyl acetate : petrol, 50:50); mp 176-179°C (from ethyl acetate and petrol), (lit.[75], 175-177°C); δ_H 1.91 (2H, m, 8-H_2), 2.46 and 2.74 (4H, m, 4-H_2, 5-H, 6-H), 3.65 (1H, m, 7-H), 3.81 (1H, t, *J* 10.5, 6-CHβ), 4.46 (1H, dd, *J* 10.5 and 4, 6-CHα), 4.94 (1H, td, *J* 7 and 4.5, 1-H), 5.51 (1H, s, O-CH-O), 7.39 (3H, m, Ar-H_3) and 7.50 (2H, m, Ar-H_2); *m/z* 260 (M^+, 44%), 259 (33), 207 (7), 183 (7), 155 (10), 154 (177), 126 (23), 125 (11), 105 (80) and 54 (100).

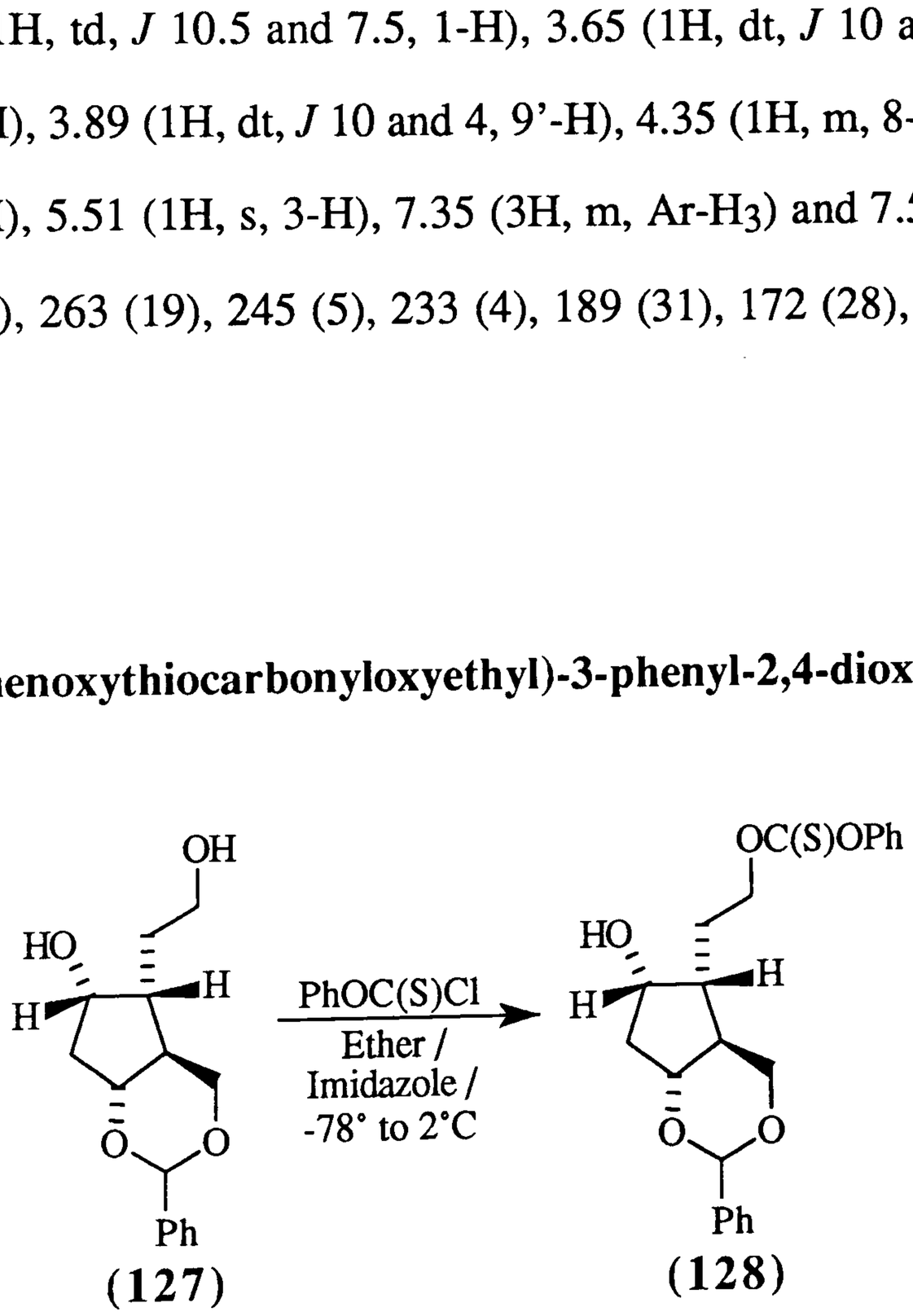
(±)-*trans*-7α-(2-Hydroxyethyl)-3-phenyl-2,4-dioxabicyclo[4.3.0]nonan-8α-ol (127)



(126) (127)

Lithium aluminium hydride (63mg, 1.67mmol) was added to THF (5ml) at 0°C. The benzylidene protected diol **(126)** (403mg, 1.54mmol) in THF (4ml) was added to the reducing system and the reactants were stirred for 0.25h at ≈(-5 to 8)°C. The reaction was quenched with saturated Rochelle salt solution, (50ml) and then extracted as usual. The protected diol **(127)** was isolated (430mg, >100% possible crude yield), but the NMR spectra, mp and MS data were as expected. R_f = 0.29 (ethyl acetate); mp 123-126°C (from ethyl acetate and petrol), (lit.[75], 129-131°C); δ_H 1.55 (2H, m, 8'-H_2), 1.72 (1H, ddd, *J* 13, 11 and 4.5, 9β-H), 1.96 (2H, m, 6-H, 7-H), 2.63 (1H, dt, *J* 13 and 7.5, 9α-H), 3.53 (1H, td, *J* 10.5 and 7.5, 1-H), 3.65 (1H, dt, *J* 10 and 2.5, 9'-H), 3.71 (1H, t, *J* 10.5, 5β-H), 3.89 (1H, dt, *J* 10 and 4, 9'-H), 4.35 (1H, m, 8-H), 4.37 (1H, dd, *J* 10.5 and 4.5, 5α-H), 5.51 (1H, s, 3-H), 7.35 (3H, m, Ar-H_3) and 7.50 (2H, m, Ar-H_2); *m/z* 264 (M^+, 17%), 263 (19), 245 (5), 233 (4), 189 (31), 172 (28), 146 (34), 121 (43) and 107 (100).

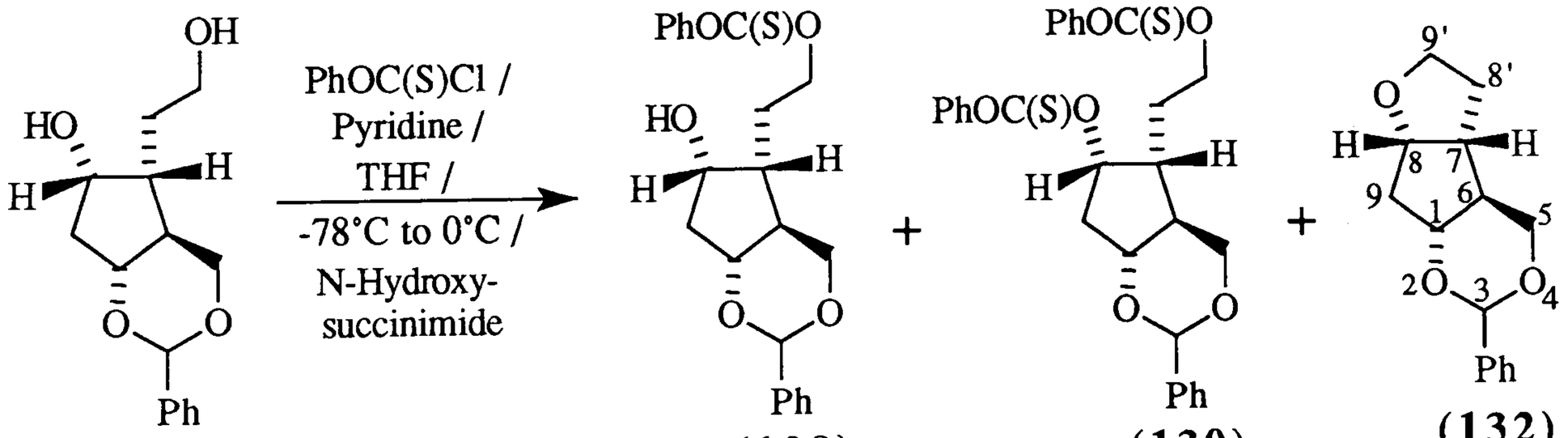
(±)-*trans*-7α-(2-Phenoxythiocarbonyloxyethyl)-3-phenyl-2,4-dioxabicyclo[4.3.0] nonan-8α-ol (128)



(127) (128)

To dried and distilled ether (50ml) was added with stirring, imidazole (476mg, 6.99mmol) and the diol **(127)** (1621mg, 6.14mmol). On cooling to -78°C, phenyl chlorothionoformate (0.90ml, 6.50mmol) was added over 0.3h, and maintained at this temperature for 1.5h. The opaque deep yellow reaction mixture was raised to -12°C for ≈12h, then allowed to attain ≈2°C for ≈12h, and recooled to -12°C and stirred for a further 7h. The usual process for extraction gave the crude product (2.88g), which was purified by column chromatography, eluting with ethyl acetate : petrol (20:80) to give the phenoxythiocarbonate **(128)**, a yellow viscous oil, (2210mg, 5.53mmol, 90%)[75]; R_f = 0.32 (ethyl acetate : petrol, 30:70); δ_H 1.60 (2H, m, 8'-H_2), 1.89 and 2.11 (3H, m, 6-H, 7-H, 9β-H), 2.29 (1H, br s, OH), 2.65 (1H, dt, *J* 13.5 and 7.5, 9α-H), 3.51 (1H, td, *J* 10 and 7.5, 1-H), 3.75 (1H, t, *J* 10.5, 5β-H), 4.26 (1H, m, 8-H), 4.43 (1H, dd, *J* 10.5 and 4.5, 5α-H), 4.58 (2H, t, *J* 6.5, 9'-H_2), 5.53 (1H, s, 3-H), 7.14 (2H, m, Ar-H_2), 7.39 (6H, m, Ar-H_6) and 7.54 (2H, m, Ar-H_2); *m/z* 400 (M^+, 1%), 307 (1), 306 (2), 294 (1), 246 (20), 140 (13), 123 (25), 107 (28), 105 (48) and 94 (100).

Attempt to optimise the preparation of (±)-*trans*-7α-(2-phenoxythiocarbonyloxyethyl)-3-phenyl-2,4-dioxabicyclo[4.3.0]nonan-8α-ol (128)



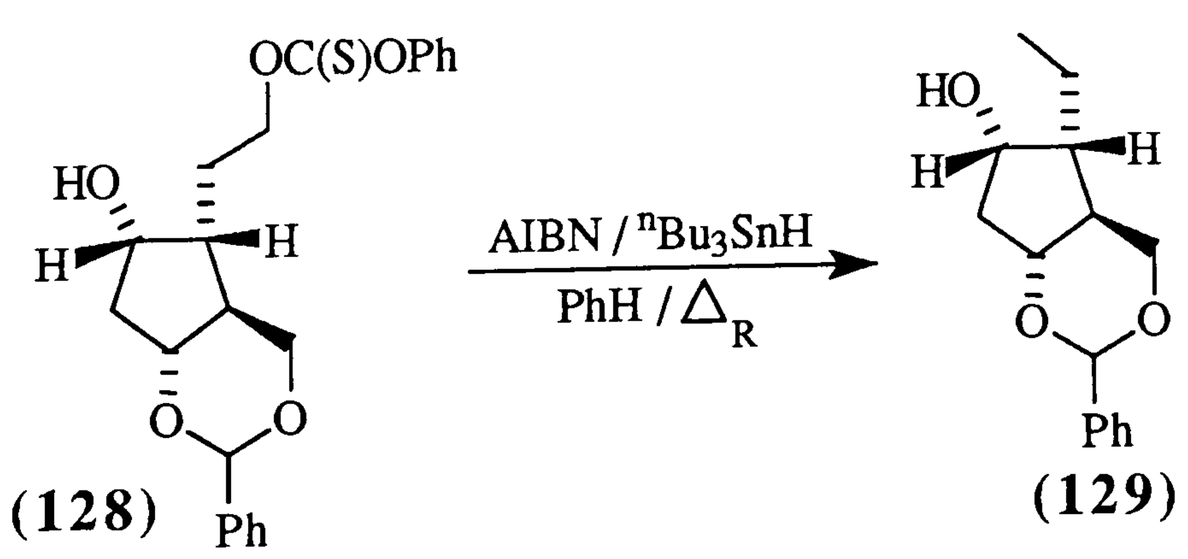
To THF (25ml) was added with stirring, pyridine (0.5ml, 6.18mmol), *N*-hydroxysuccinimide (catalytic amount) and the diol **(127)** (430mg, 1.63mmol) and the mixture was cooled to -78°C. Phenyl chlorothionoformate (0.3ml, 2.17mmol) was added over 0.1h. The opaque deep yellow reaction mixture attained 0°C over 3h, and by TLC analysis some of the desired product and some diphenoxythiocarbonate ester were formed, but no starting material remained. The usual extraction process followed. The crude extract was purified by column chromatography, eluting initially with ethyl

acetate : petrol (10:90), to give (±)-*trans*-8α-(phenoxythiocarbonyloxy)-7α-(2-phenoxythiocarbonyloxyethyl)-3-phenyl-2,4-dioxabicyclo[4.3.0]nonane (**130**) (484mg, 0.902mmol, 55%); R_f = 0.68 (ethyl acetate : petrol, 30:70); (M^+ not found); υ_{max}/cm^{-1} 1204 and 1277 (2 x C=S); δ_H 2.11 (5H, m, 6-H, 7-H, 8'-H_2 and 9β-H), 2.95 (1H, dt, *J* 14.5 and 7.5, 9α-H), 3.65 (1H, td, *J* 10 and 8, 1-H), 3.83 (1H, t, *J* 10.5, 5β-H), 4.50 (1H, dd, *J* 10.5 and 4, 5α-H), 4.56 (2H, m, 9'-H_2), 5.56 (1H, s, 3-H), 5.63 (1H, m, 8-H), 7.11 (4H, m, Ar-H_4), 7.26, (3H, m, Ar-H_3), 7.38 (6H, m, Ar-H_6) and 7.53 (2H, m, Ar-H_2); *m/z* (CI) 535 ([M-H]$^+$, 1%), 459 (1), 441 (2), 432 (2), 431 (9), 413 (4), 337 (28), 295 (8), 277 (92), 259 (98), 139 (37) and 105 (100); molecular ion not found.

Further elution with ethyl acetate : petrol (20:80) gave the colourless liquid cyclic ether (**132**) (24mg, 0.0976mmol, 6%); R_f = 0.28 (ethyl acetate : petrol, 25:75); (Found M^+, 246.1257. $C_{15}H_{18}O_3$ requires M, 246.1256); δ_H 1.77 and 2.17 (5H, m, 6-H, 7-H, 8'-H_2, 9β-H), 2.53 (1H, dt, *J* 12 and 7, 9α-H), 3.50 (1H, ddd, *J* 12, 10.5 and 6.5, 1-H), 3.78 (1H, t, *J* 10.5, 5β-H), 3.89 (2H, m, 9'-H_2), 4.42 (1H, dd, *J* 10.5 and 4, 5α-H), 4.47 (1H, m, 8-H), 5.50 (1H, s, 3-H), 7.10 (3H, m, Ar-H_3) and 7.41 (2H, m, Ar-H_2); δ_C 30.11 (C-8'), 37.00 (C-9), 41.03 and 45.61 (C-6, C-7), 67.38 (C-9'), 72.13 (C-5), 79.94 (C-8), 80.94 (C-1), 101.99 (C-3) and 126.24 128.34 128.99 and 137.99 (C-aromatics); *m/z* 246 (M^+, 23%), 245 (14), 140 (11), 139 (19), 123 (26) 105 (38) and 94 (100).

Lastly, elution with ethyl acetate : petrol (25:75), returned the desired phenoxythiocarbonate ester (**128**) (180mg, 0.450mmol, 28%); spectral data as before.

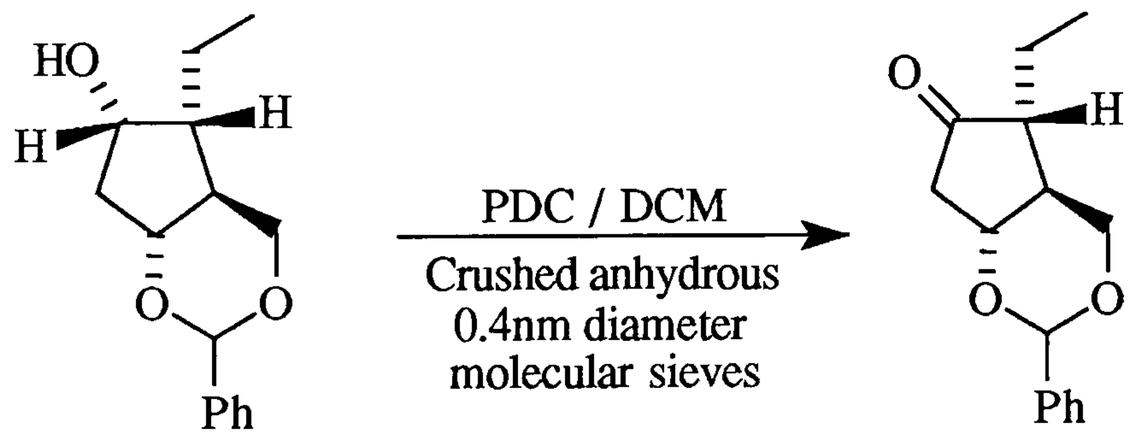
(±)-*trans*-7α-Ethyl-3-phenyl-2,4-dioxabicyclo[4.3.0]nonan-8α-ol (129)



Phenoxythiocarbonate (**128**) (172.5mg, 0.431mmol) in dried benzene (45ml) was heated to reflux for 0.2h under nitrogen. AIBN (catalytic quantity) was added

followed, under continuing reflux, by tri-*n*-butyltin hydride (0.13ml, 0.483mmol) over 0.1h. Characteristic bubbling of this reaction was seen, and heated to reflux for 3h. The reaction mixture was evaporated *in vacuo*. Purification by column chromatography, initially eluting with ethyl acetate : petrol (5:95) to remove tin-containing compounds, then with 20:80 eluting the alcohol **(129)** as a dry white flaky solid, (80mg, 0.323mmol, 75%); R_f = 0.60 (ethyl acetate : petrol, 50:50); mp 84-86°C (from carbon tetrachloride and petrol), (lit.[75], 89-91°C); δ_H 0.97 (3H, t, *J* 7.5, 9'-H_3), 1.45 (5H, m, 8'-H_2, 7-H, 9β-H, 6-H), 2.67 (1H, dt, *J* 13.5 and 7, 9α-H), 3.51 (1H, td, *J* 10 and 7.5, 1-H), 3.76 (1H, t, *J* 10.5, 5β-H), 4.31 (1H, m, 8-H), 4.45 (1H, dd, *J* 10.5 and 4, 5α-H), 5.52 (1H, s, 3-H), 7.36 (3H, m, Ar-H_3) and 7.51 (2H, m, Ar-H_2); *m/z* 248 (M^+, 14%), 247 (15), 124 (9), 121 (10), 108 (12), 107 (100) and 105 (44).

(±)-*trans*-7α-Ethyl-3-phenyl-2,4-dioxabicyclo[4.3.0]nonan-8-one (142)

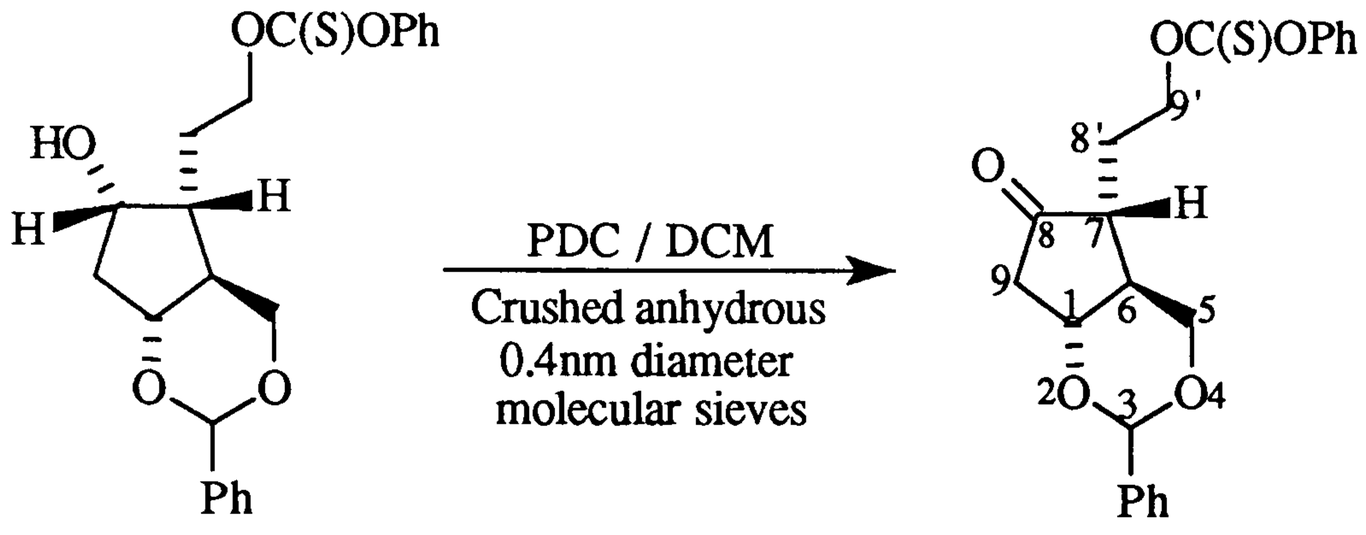


(129) **(142)**

To DCM (5ml) under an inert atmosphere were added anhydrous crushed molecular sieves of 0.4nm diameter (approximately 0.5g) and pyridinium dichromate (70mg, 0.185mmol). Alcohol **(129)** (30mg, 0.121mmol) in DCM (5ml) and added dropwise over 0.25h. The reaction mixture was allowed to stir for 2.25h at room temperature, during which time the suspension blackened. The contents of the flask were then filtered through anhydrous magnesium sulfate, Florisil® (magnesium silicate 60-100 mesh) and Celite®, and the reaction flask rinsed with DCM (8 x 10ml), and likewise passed through the filter system; the combined organic phases were evaporated *in vacuo*. The crude cyclopentanone **(142)** was a white solid, which did not require further purification (22mg, 0.894mmol, 74%); R_f = 0.71 (ethyl acetate : petrol, 30:70); mp 71-72°C (from ethyl acetate and petrol), (lit.[75], 70-72°C); δ_H 0.95 (3H, t, *J* 7.5, 9'-H_3), 1.73 and 2.17 (4H, m, 6-H, 7-H, 8'-H_2), 2.43 (1H, dd, *J* 17.5 and 11.5, 9β-H), 2.73

(1H, dd, *J* 17.5 and 7, 9α-H), 3.97 (1H, t, *J* 10.5, 5β-H), 4.04 (1H, m, 1-H), 4.53 (1H, dd, *J* 10.5 and 4, 5α-H), 5.74 (1H, s, 3-H), 7.37 (3H, m, Ar-H_3) and 7.52 (2H, m, Ar-H_2); *m/z* 246 (M^+, 32%), 245 (17), 177 (12), 155 (11), 120 (10), 112 (14), 107 (100) and 105 (70).

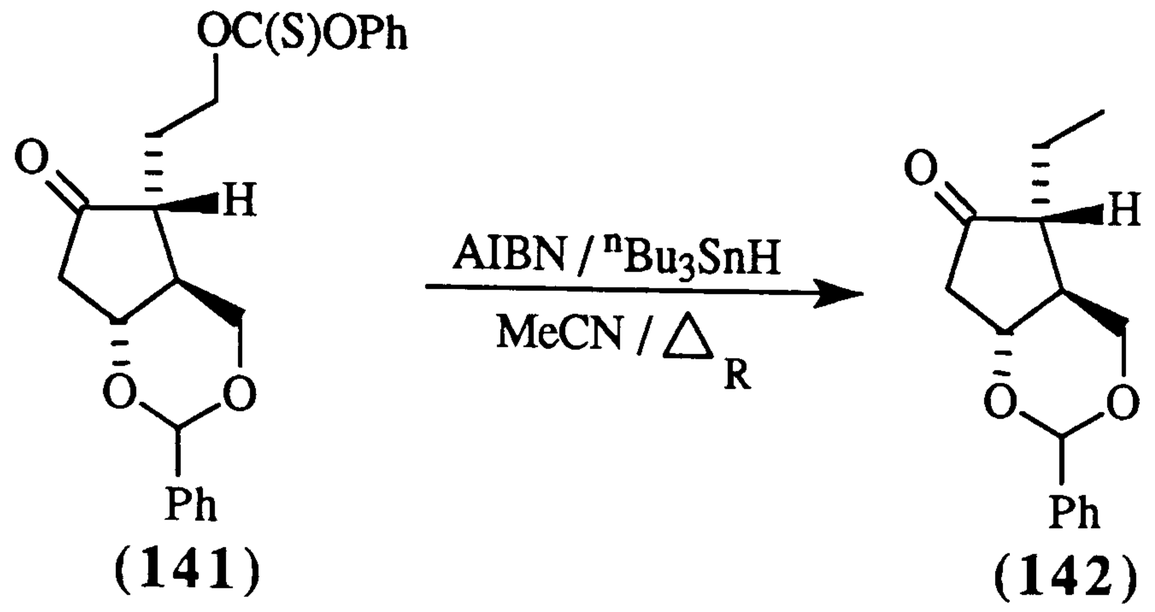
(±)-*trans*-7α-(2-Phenoxythiocarbonyloxyethyl)-3-phenyl-2,4-dioxabicyclo[4.3.0] nonan-8-one (141)



(128) **(141)**

The phenoxythiocarbonate **(128)** (400mg, 1.00mmol) was treated in the same way as the cyclopentanol **(129)** with PDC (624mg, 1.65mmol) and anhydrous crushed molecular sieves to give a pale cream solid (333mg, 0.837mmol, 84%). Crystallisation from carbon tetrachloride and petrol gave the ketone **(141)** (174mg, 0.437mmol, 44%) as a white solid; a further 121mg (0.304mmol, 30%) was isolated from the filtrate; thus a total of 295mg, 0.741mmol, 74% was obtained; R_f = 0.53 (ethyl acetate : petrol, 30:70); mp 116-117°C (from carbon tetrachloride and petrol); (Found C, 66.4; H, 5.94; S, 7.78%; M^+, 398.1175. $C_{22}H_{22}O_5S$ requires C, 66.3; H, 5.56; S, 8.05%; M, 398.1188); υ_{max}/cm^{-1} 1202 (C=S) and 1699 (C=O); δ_H 2.18 (4H, m, 6-H, 7-H, 8'-H_2), 2.51 (1H, dd, *J* 17 and 11, 9β-H), 2.81 (1H, dd, *J* 17 and 7, 9α-H), 3.97 (1H, t, *J* 11, 5β-H), 4.05 (1H, m, 1-H), 4.59 (1H, dd, *J* 11 and 4, 5α-H), 4.61 (2H, m, 9'-H_2), 5.71 (1H, s, 3-H), 7.10 (2H, m, Ar-H_2), 7.29 (1H, m, Ar-H), 7.38 (5H, m, Ar-H_5) and 7.52 (2H, m, Ar-H_2); δ_C 26.44 (C-8'), 43.09 (C-9), 45.60 and 47.70 (C-6, C-7), 71.34 and 71.78 (C-5, C-9'), 78.41 (C-1), 102.53 (C-3), 121.88, 126.12, 126.67, 128.41, 129.23, 129.58, 137.46 and 153.31 (aromatics), 194.85 (C-5) and 211.08 (C-8); *m/z* 398 (M^+, 2%), 292 (2), 247 (3), 246 (20), 245 (100), 141 (79), 139 (38), 138 (17), 105 (45) and 97 (87).

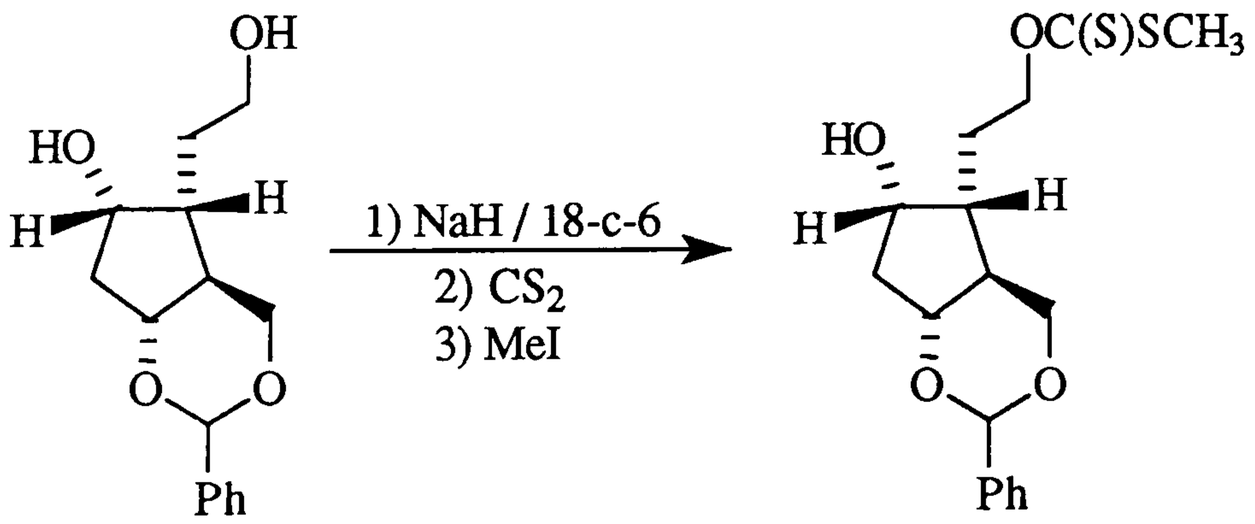
Reduction of keto-thiocarbonate (141) to form the cyclopentanone (142)



(141) **(142)**

The ketone **(141)** (267mg, 0.671mmol) was heated to reflux with $^{n}Bu_3SnH$ (0.23ml, 0.855mmol) and AIBN (≈30mg) in MeCN (50ml) for 6.25h. On cooling, petrol (≈50ml) was added and the biphasic system stirred for ≈14h. The liquids were separated, the acetonitrile portion extracted with more petrol (2 x ≈50ml) and the acetonitrile evaporated *in vacuo*. Purification by column chromatography (eluting with ethyl acetate : petrol, 10:90) gave **(142)**, spectral data as previously reported (61mg, 0.248mmol, 37%).

(±)-*trans*-7α-(Methyldithiocarbonyloxyethyl)-3-phenyl-2,4-dioxabicyclo[4.3.0] nonan-8α-ol (143)

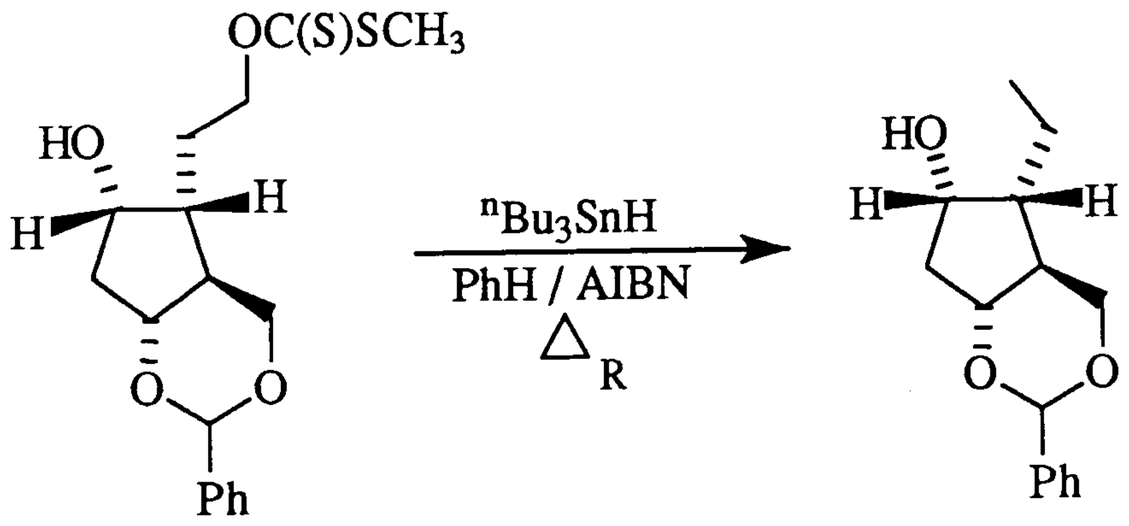


(127) **(143)**

Sodium hydride (27mg, ≡16.4mgNaH, 0.674mmol, 60% dispersed in mineral oil) was washed twice with hexane, and THF (10ml) added plus 18-crown-6 (catalytic quantity) and stirred for 0.4h, then cooled to 0°C. Diol **(127)** (160mg, 0.606mmol) in THF (30ml) was added to the reaction mixture, allowed to reach room temperature and stirred for 0.5h. To the reaction was added carbon disulfide (0.038ml, 0.632mmol) and stirred for 2.25h. Finally, methyl iodide (0.039ml, 0.626mmol) was added. After 2h, no starting material was apparent by TLC analysis, so the usual extraction procedure was performed. The impure product **(143)** (201mg, 0.568mmol, 94%) was an orange liquid. Crude xanthate **(143)**

(104mg 0.294mmol) was used in an impure state in a subsequent reaction, whilst 97mg was purified by column chromatography (eluting with ethyl acetate : petrol, 30:70) to give a pale orange liquid (**143**) (36mg, 37% of contaminated product added to flash chromatography column); R_f = 0.44 (ethyl acetate : petrol, 30:70); (M^+ absent; Found $[MH-108]^+$, 247.1335. $C_{17}H_{23}O_4S$ (MH-108 (-$CH_3SC(S)OH$)) requires, 247.1334); υ_{max}/cm^{-1} 700 (C-S) and 1226 (C=S); δ_H 1.54 (1H, m, 8'-H), 1.69 (1H, ddd, *J* 13.5, 10.5 and 4, 6-H), 1.85 (1H, m, 8'-H), 1.94 (1H, br s, 8-OH), 1.99 (1H, m, 9β-H), 2.16 (1H, m, 7-H), 2.58 (3H, s, CH_3), 2.67 (1H, dt, *J* 13.5 and 7.5, 9α-H), 3.51 (1H, td *J* 10.5 and 7.5, 1-H), 3.74 (1H, t, *J* 10.5, 5β-H), 4.29 (1H, m, 8-H), 4.41 (1H, dd, *J* 10.5 and 4, 5α-H), 4.67 (2H, t, *J* 6.5, 9'-H_2), 5.51 (1H, s, 3-H), 7.36 (3H, m, Ar-H_3) and 7.50 (2H, m, Ar-H_2); δ_C 19.18 (CH_3), 25.43 (C-8'), 39.59 (C-9), 40.42 and 43.59 (C-6, C-7), 69.27 (C-8), 71.97 (C-9'), 72.99 (C-5), 80.96 (C-1), 102.07(C-3), 126.26, 128.29, 128.99 and 138.01 (aromatics) and 216.01 (C=S); *m/z* (CI) 355 $[MH]^+$, (41%), 307 (5), 248 (15), 247 (71), 246 (9), 245 (14), 231 (15), 169 (23), 141 (55) and 123 (100).

Reduction of xanthate (143) to form the cyclopentanol (129)

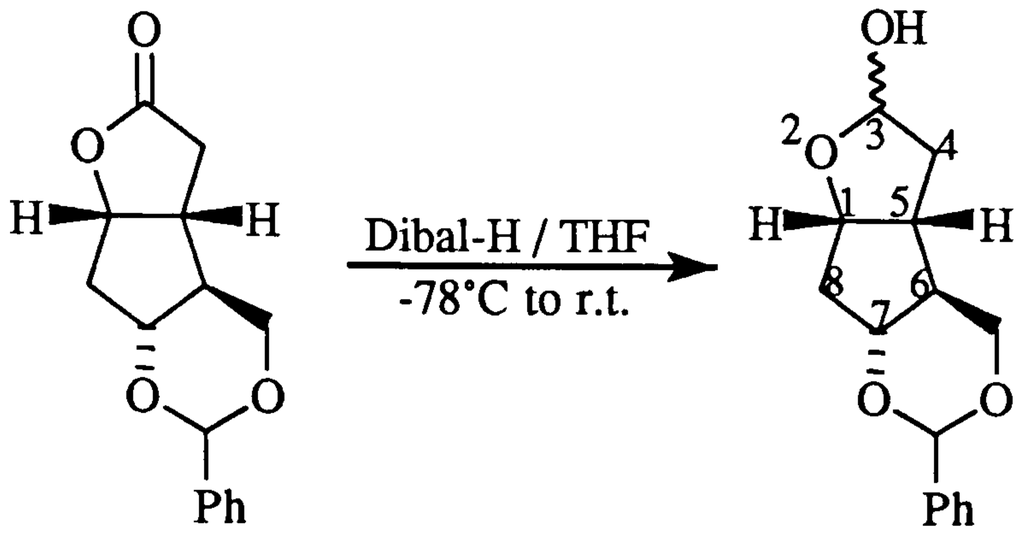


(143) **(129)**

The crude xanthate (**143**) (104mg, 0.294mmol) was reacted with tri-*n*-butyl tin hydride (0.15ml, 0.558mmol) and AIBN (catalytic quantity) in benzene (25ml) at reflux for 2.5h, by the same manner as the phenoxythiocarbonate (**128**). Post column chromatography, the ethyl derivative (**129**) was obtained, data as previously reported (29mg, 0.117mmol, 40%). Treatment of (**143**) with triphenyl tin hydride returned the cyclopentanol (**129**) in 43% yield after purification by column chromatography.

The column-chromatographed xanthate **(143)** (35mg, 0.0989mmol) was likewise treated, but post purification returned less product, **(129)** (3mg, 0.0121mmol, 12%).

Synthesis of the benzylidene derivative of (±)-*cis*-7α-hydroxy-6β-hydroxymethyl-2-oxabicyclo[3.3.0]octan-3ξ-ol (149)

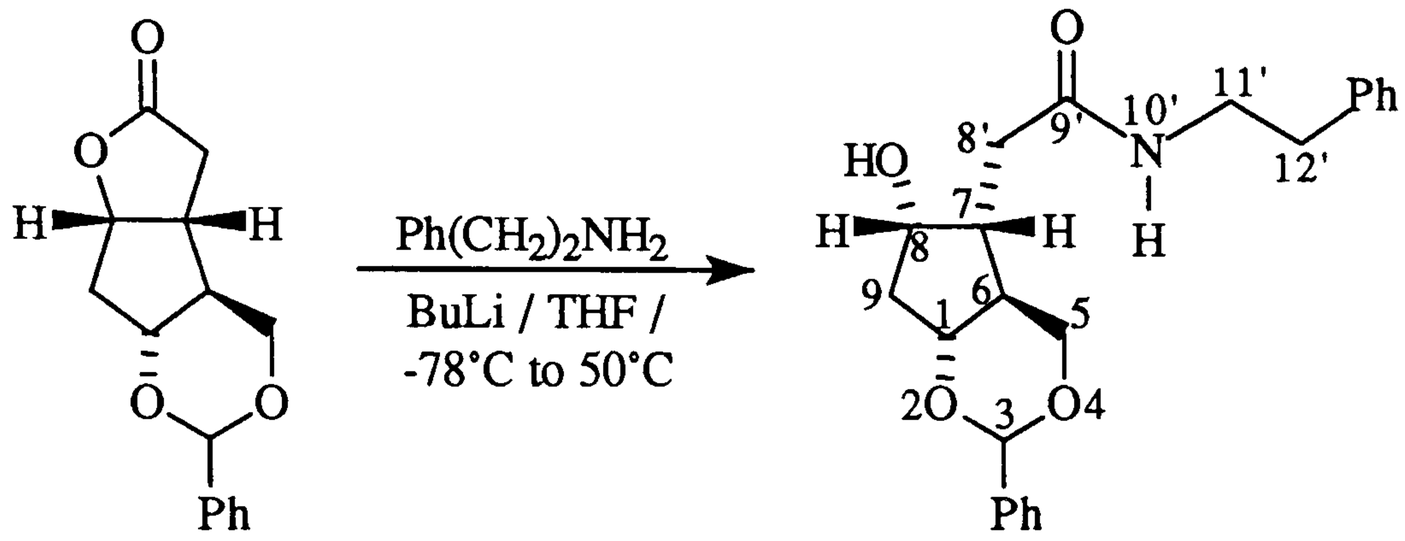


(126) **(149)**

To a stirred solution of the lactone **(126)** (140mg, 0.538mmol) in THF (20ml) at -78°C was added Dibal-H (0.55ml of 1M solution, 0.55mmol) over 0.1h. The reaction mixture was maintained at -78°C for 0.5h, then the temperature was allowed to rise to room temperature over 3.75h. As no change in TLC value from starting material was visible at any stage, the reaction was quenched with Rochelle salt solution (5ml), and extracted as usual. The residue was purified by column chromatography eluting with ethyl acetate : petrol (50:50), yielding the lactol **(149)** (120mg, 0.458mmol, 74%), as a white solid, which exists as two isomers in 58:42 ratio, but also contains approximately 11% starting material, which proved impossible to separate. R_f = 0.42 (ethyl acetate : petrol, 65:35); (Found: M^+, 262.1199. $C_{15}H_{18}O_4$ requires M, 262.1206); υ_{max}/cm^{-1} * 780 (C-O, acetal), 959 (C-O, lactol) and 3398 (O-H). Major isomer, δ_H 1.91 (2H, m, 8-H_2), 2.27 and 2.64 (4H, m, 4-H_2, 5-H, 6-H), 3.55 (1H, m, 7-H), 3.76 (1H, t, *J* 10.5, 6-$CH_2\beta$), 4.42 (1H, dd, *J* 10.5 and 4, 6-$CH_2\alpha$), 4.67 (1H, m, 1-H), 5.48 (1H, s, O-CH-O), 5.70 (1H, dd, *J* 5 and 1.5, 3-H), 7.36 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2); δ_C 36.36 (C-8), 37.49 (C-4), 39.76 (C-5), 46.92 (C-6), 71.70 (6-CH_2), 79.08 (C-1), 81.83 (C-7), 100.49 and 101.72 (O-CH-O, C-3) and 126.23, 128.27, 128.89 and 137.80 (aromatics). Minor isomer, δ_H 1.91 (2H, m, 8-H_2), 2.27 and 2.64 (4H, m, 4-H_2, 5-H, 6-H), 3.55 (1H, m, 7-H), 3.76 (1H, t, *J* 10.5, 6-$CH_2\beta$), 4.42 (1H, dd, *J* 10.5 and 4, 6-$CH_2\alpha$), 4.67 (1H, m, 1-H), 5.49 (1H, s, O-CH-O), 5.64 (1H, m, 3-H), 7.36 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2); δ_C 36.46 (C-8), 39.03 (C-4), 40.11 (C-5), 45.49 (C-6), 72.17 (C-9), 81.18 (C-1), 81.40 (C-7), 101.07 (C-10), 101.86 (C-3) and 126.15, 128.21, 128.89 and 137.80

(aromatics). *m/z* 262 (M^+, 41%), 261 (23), 260 (10), 244 (2), 190 (10), 156 (4), 121 (4), 108 (17), 107 (100) and 105 (50).

(±)-*trans*-3-Phenyl-7α-(N-(2-phenylethyl)acetamido)-2,4-dioxabicyclo[4.3.0]nonan-8α-ol (150)

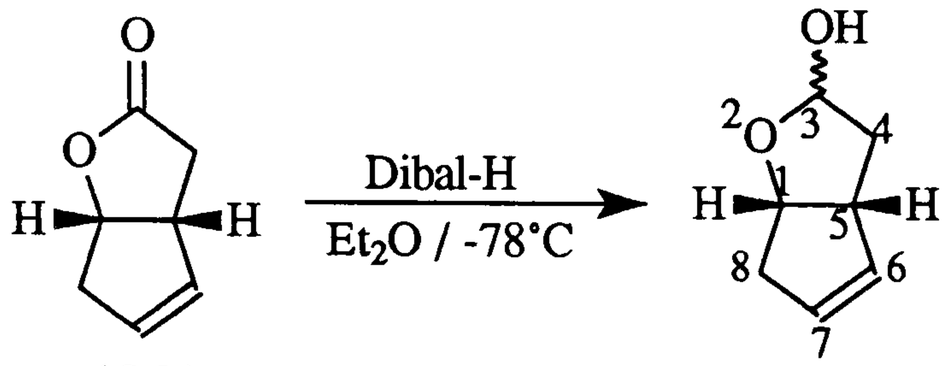


(126) **(150)**

n-Butyl lithium (0.5ml of a 2.5M solution in hexanes, 1.25mmol) was added to β-phenylethylamine (1.5ml, 1.19mmol) in THF (20ml) at -78°C, stirred for 0.5h and allowed to warm to 8°C, giving a deep orange-red coloured solution of the amide anion. The temperature was lowered to -78°C, and lactone **(126)** (260mg, 1.00mmol) added. The reaction was allowed to reach room temperature and maintained for 3.25h at this temperature. As no difference compared to starting material was noted by TLC, the reaction was heated to 50°C for 15h; however, again no change in R_f was noted, thus the reaction was cooled and extracted. Flash chromatography with ethyl acetate yielded the amide **(150)**, (223mg, 0.585mmol, 59%), an off-white solid; R_f = 0.51 (100% ethyl acetate); mp 180-181°C (from carbon tetrachloride, chloroform and petrol); (Found C, 72.2; H, 7.20; N 3.67%; M^+, 381.1942. $C_{23}H_{27}NO_4$ requires C, 72.4; H, 7.13; N, 3.67%; M, 381.1940); υ_{max}/cm^{-1} 1559 and 1555 (N-H, bending), 1617 (C=O), 3322 (N-H) and 3404 (OH); δ_H 1.77 and 1.88 (3H, m, 6-H, 7-H, 9β-H), 2.13 (1H, dd, *J* 14 and 3.5, 8'-H), 2.58 (2H, m, 12'-H_2), 2.77 (1H, m, 9α-H), 2.79 (1H, m, 8'-H), 2.97 (1H, m, 8-OH), 3.53 (3H, m, 1-H, 11'-H_2), 3.69 (1H, t, *J* 10.5, 5β-H), 4.28 (1H, dd, *J* 10.5 and 4, 5α-H), 4.31 (1H, m, 8-H), 5.49 (1H, s, 3-H), 5.76 (1H, br s, N-H), 7.28 (2H, m, Ar-H_2), 7.33 (6H, m, Ar-H_6) and 7.49 (2H, m, Ar-H_2); δ_C 34.36, 35.46 and 38.81 (C-8', C-11', C-12'), 40.41 (C-7), 40.57 (C-9), 44.21 (C-6'), 68.94 (C-8), 71.71 (C-5), 80.54 (C-

1), 102.08 (C-3), 126.26, 126.65, 128.27, 128.70, 128.80, 128.94, 138.02 and 138.54 (aromatics) and 172.89 (C-9'); *m/z* 381 (M^+, 44%), 275 (7), 261 (16), 259 (14), 246 (11) 219 (63), 203 (11), 202 (16), 163 (35) and 104 (100).

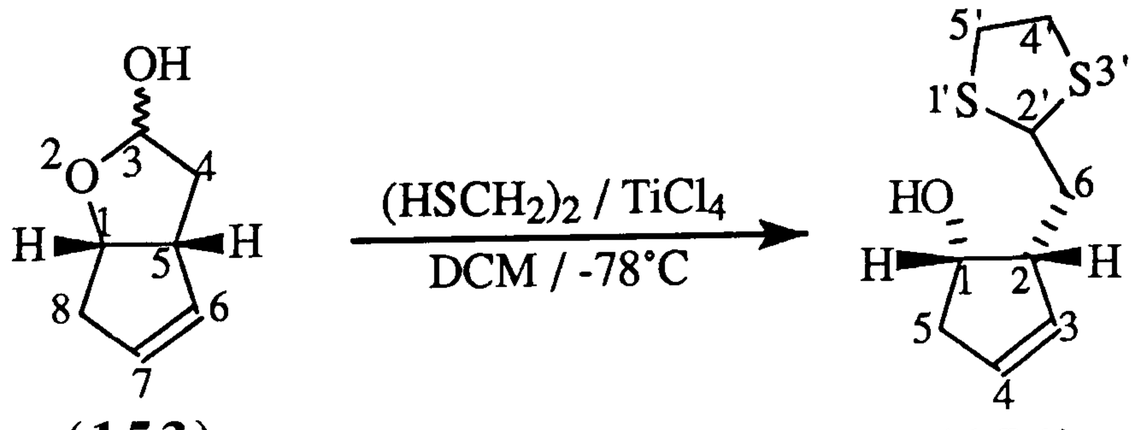
(±)-*cis*-2-Oxabicyclo[3.3.0]oct-6-en-3ξ-ol (153)



(82) **(153)**

Dibal-H (1.3ml of a 1.0M solution, 1.30mmol) was added to lactone **(82)** (149mg, 1.20mmol) in ether (4ml) at -78°C. The reaction was stirred at -78°C for 3.5h, then quenched with water, acidified with 1M sulfuric acid (2ml) and extracted as normal except using ether. Purification by column chromatography, eluting with ethyl acetate : petrol (30:70), yielded the lactols **(153)**, (125mg, 0.992mmol, 82%), as two isomers in a ratio of 66:34; R_f = 0.42 (ethyl acetate:petrol, 25:75)[119]; υ_{max}/cm^{-1} 3387 (O-H). δ_H Major isomer, 1.82, 2.10 and 2.56 (4H, m, 4-H_2, 8-H_2), 3.35 (1H, m, 5-H), 4.78 (1H, m, 1-H), 5.41 (1H, m, 3-H), 5.55 (2H, m, 6-H, 7-H) and 5.78 (1H, m, O-H); minor isomer, δ_H 1.82, 2.10 and 2.56 (4H, m, 4-H_2, 8-H_2), 3.35 (1H, m, 5-H), 4.78 (1H, m, 1-H), 5.26 (1H, m, 3-H), 5.55 (2H, m, 6-H, 7-H) and 5.78 (1H, m, O-H). *m/z* 126 (M^+, 2%), 125 (13), 109 (100), 108 (11), 91 (11), 81 (40) and 79 (19).

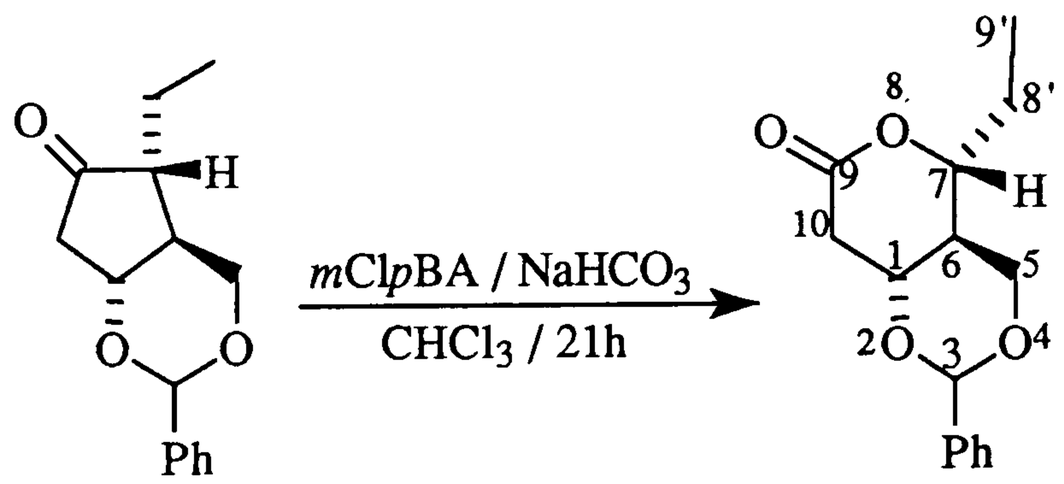
(±)-2α-1',3'-Dithiolan-2'-yl-methyl-cyclopent-3-en-1α-ol (154)



(153) **(154)**

To lactol **(153)** (19.8mg, 0.157mmol) in DCM (4ml) at -78°C was added ethane-1,2-dithiol (0.25ml, 0.298mmol) with stirring, then $TiCl_4$ (0.25ml of a 1M solution in DCM, 0.25mmol) added over 0.05h, to give a moderately strong yellow coloured solution. After 0.5h at -78°C., the impure product was extracted as usual, except using ether (30ml). The residue was purified by column chromatography eluting with ethyl acetate : petrol (10:90), yielding the dithiane **(154)**, a pale grey gelatinous solid, (23mg, 0.114mmol, 72%); R_f = 0.54 (ethyl acetate : petrol, 30:70); (Found: $[M\text{-}H]^+$, 201.0416. $C_9H_{14}OS_2$ requires M-H^+, 201.0408); δ_H 2.01 (1H, dt, *J* 14 and 7.5, 6-H), 2.18 (1H, ddd, *J* 14, 8.5 and 7.5, 6-H), 2.36 (1H, dddd, *J* 17, 6, 3.5 and 1.5, 5-H), 2.66 (1H, dddd, *J* 17, 5.5, 3 and 2, 5-H), 2.78 (1H, m, 2-H), 3.28 (4H, m, 4'-H_2 and 5'-H_2), 4.45 (1H, m, 1-H), 4.66 (1H, t, *J* 7.5, 2'-H) and 5.63 and 5.78 (1H, m, 3-H, 4-H); δ_C 37.63, 38.32, 38.38 and 42.11 (C-5, C-6, C-4', C-5'), 49.97 and 52.48 (C-2, C-2'), 72.41 (C-1) and 128.37 and 132.45 (C-4', C-5'); *m/z* 202 (M^+, 2%), 201 (36), 200 (19), 184 (39), 174 (20), 141 (24), 124 (35), 109 (100), 105 (81), 91 (11), 83 (84) and 81 (64).

(±)-*trans*-7α-Ethyl-3-phenyl-2,4,8-trioxabicyclo[4.4.0]decan-9-one (156)

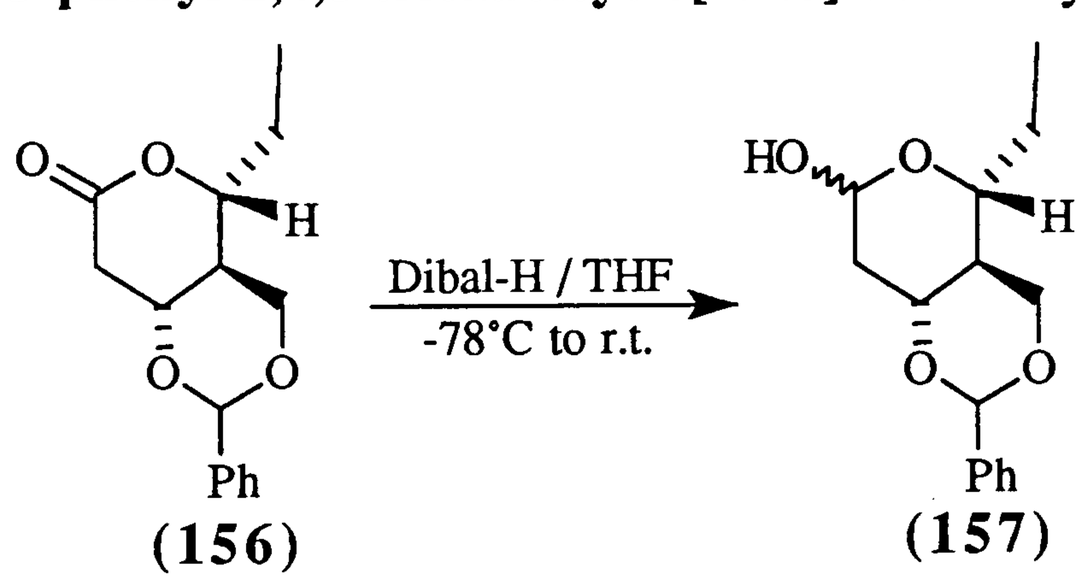


(142) **(156)**

The cyclopentanone **(142)** (253mg, 1.03mmol) was dissolved in chloroform (60ml), and sodium hydrogen carbonate (≈9g, ≈100mmol) added with stirring. Non-recrystallised *m*Cl*p*BA (3.08g, approximately 50% oxidant ≡ 1.54g per-acid, 8.92mmol) was added over 0.35h. The reaction was stirred for 21h at room temperature, forming a gelatinous suspension. Water (20ml) and saturated aqueous sodium sulfite (20ml) were added, and the organic layer separated; the aqueous layer was further extracted with chloroform (4 x 20ml). The combined organic layers were washed with aqueous saturated sodium hydrogen carbonate and sodium sulfite solution (≈1:1, 10ml),

followed by brine (20ml); then dried over anhydrous sodium sulfate, filtered and evaporated, (558mg, >100%). The δ-valerolactone was recrystallisedised from carbon tetrachloride and petrol (181mg) and the remaining product in the contaminated filtrate was purified by column chromatography, eluting with ethyl acetate : petrol (20:80) to obtain a further 28mg, thus a total yield consisting 209mg, (0.798mmol, 78%) of the white flaky powder (**156**); R_f = 0.34 (ethyl acetate : petrol, 30:70); mp 132-134°C (from ethyl acetate and petrol), (lit.[75], 130-131°C); δ_H 1.08 (3H, t, *J* 7.5, 9'-H_3), 1.57 and 1.76 (2H, m, 8'-H_2), 2.07 (1H, qd, *J* 11 and 5, 6-H), 2.67 (1H, dd, *J* 18 and 11.5, 10β-H), 3.08 (1H, dd, *J* 18 and 6, 10α-H), 3.70 (1H, t, *J* 11, 5β-H), 4.08 (2H, m, 1-H and 7-H), 4.29 (1H, dd, J 11 and 5, 5α-H), 5.57 (1H, s, 3-H), 7.37 (3H, m, Ar-H_3) and 7.47 (2H, m, Ar-H_2); *m/z* 262 (M^+, 83%), 261 (100), 185 (8), 175 (9), 127 (8), 107 (21), 106 (16) and 105 (84).

(±)-*trans*-7α-Ethyl-3-phenyl-2,4,8-trioxabicyclo[4.4.0]-decan-9ξ-ol (157)

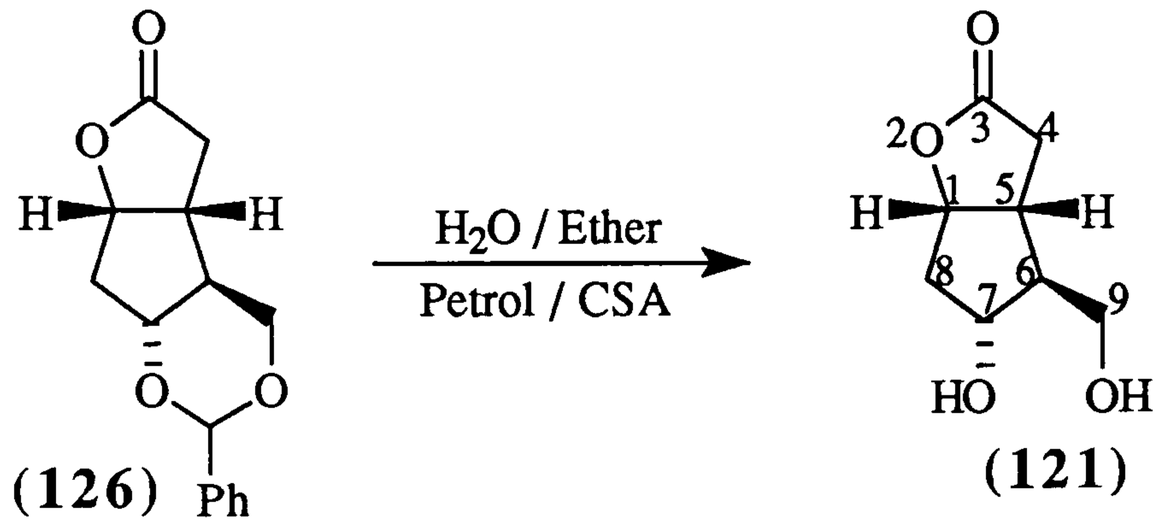


(156) **(157)**

To a stirred solution of the lactone (**156**) (87mg, 0.332mmol) in THF (8ml) at -78°C was added Dibal-H (0.38ml of a 1M solution, 0.38mmol) over 0.1h. The temperature was allowed to rise to 3°C over 2.25h. As no change in TLC value from starting material was apparent, the reaction was allowed to attain room temperature over a further 2h. Rochelle salt solution (5ml) and water (5ml) were added and the products (**157**) extracted and dried by the standard technique to give a mixture of two isomers in a ratio of 71:29 (83mg, 0.314mmol, 95%); R_f = 0.33 (ethyl acetate : petrol, 30:70); mp 112-113°C (from DCM and petrol), (lit.[75], 114.5-115.5°C). δ_H major isomer, 0.96 (3H, t, *J* 7.5, 9'-H_3), 1.52 and 1.84 (4H, m, 6-H, 8'-H_2, 10β-H), 2.13 (1H, ddd, *J* 12.5, 4.5 and 1, 10α-H), 3.07 (1H, br s, OH), 3.65 (1H, t, *J* 11, 5β-H), 3.70 (1H, m, 7-H), 4.17 (1H,

dd, *J* 11 and 4.5, 5α-H), 4.22 (1H, m, 1-H), 5.45 (1H, br s, 9-H), 5.60 (1H, s, 3-H), 7.36 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2); minor isomer, δ_H 1.00 (3H, t, *J* 7.5, 9'-H_3), 1.56 and 1.84 (4H, m, 6-H, 8'-H_2, 10β-H), 2.29 (1H, ddd, *J* 12, 4.5 and 1, 10α-H), 3.07 (1H, s, OH), 3.58 (1H, t, *J* 11, 5β-H), 3.73 (2H, m, 1-H, 7-H), 4.19 (1H, m, 5α-H), 4.83 (1H, br d, *J* 9, 9-H), 5.55 (1H, s, 3-H), 7.36 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2). *m/z* (CI) 265 ([MH]$^+$, 16%), 264 (71), 263 (60), 217 (8), 187 (5), 141 (7), 123 (23), 107 (69), 105 (69), 95 (26), 85 (52) and 83 (100).

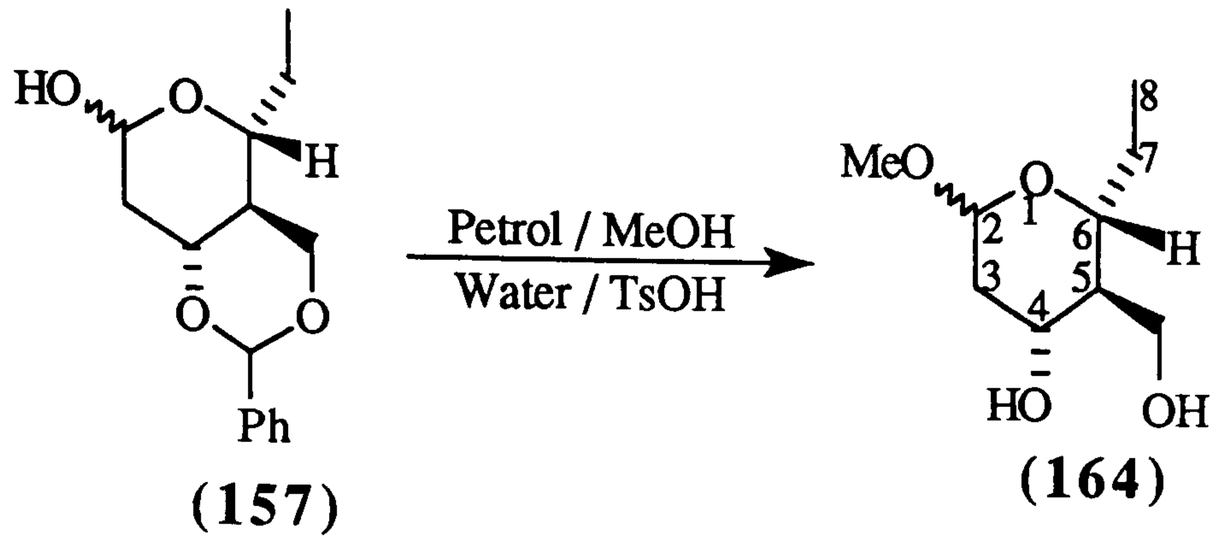
Treatment of (126) with camphor sulfonic acid, water, ether and petrol



(126) **(121)**

To benzylidene protected lactone **(126)** (36mg, 0.209mmol) dissolved in water (4ml), ether (1ml) and petrol (4ml) was added CSA (catalytic quantity); the reaction was stirred for 48h at room temperature. By TLC analysis no starting material was present, only a more polar product. Petrol (2 x 5ml) and water (2 x 5ml) were added. The bilayer system was mixed, separated, and the aqueous layer further extracted with petrol (10ml). The combined organic portions were washed with water (5ml); and the accumulated aqueous fractions were made alkaline to *p*H≈8 with solid aqueous sodium hydrogen carbonate. The water was evaporated *in vacuo* , and the resultant solid was purified by column chromatography using methanol : ethyl acetate (10:90), to give the diol **(121)** (22mg, 0.128mmol, 92%); spectral data as previously reported.

(±)-6α-Ethyl-4α-hydroxy-5β-hydroxymethyl-2ζ-methoxy-1-oxacyclohexane (164)

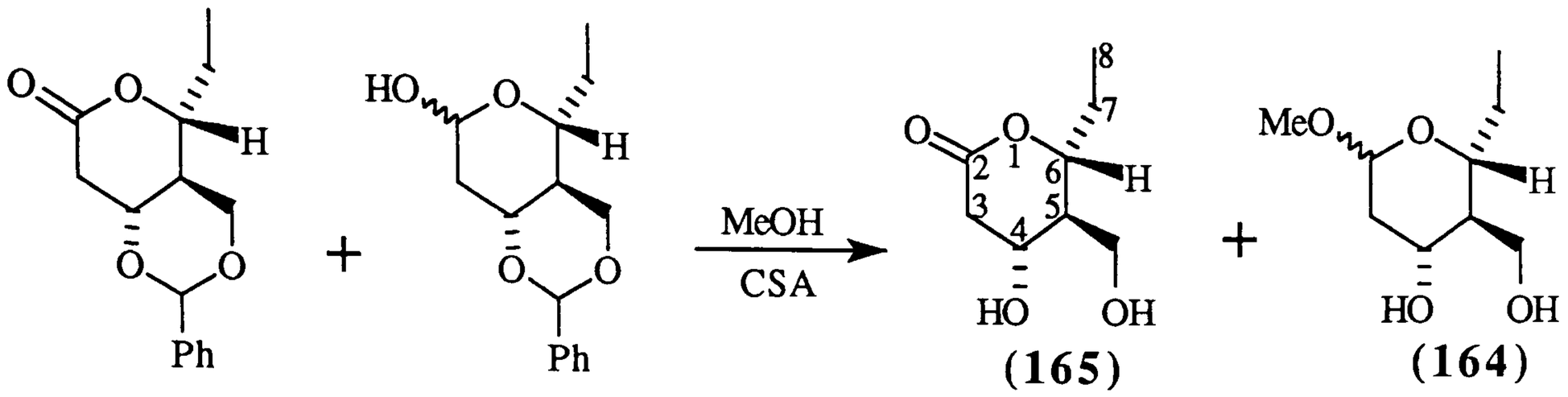


(157) **(164)**

The lactol (**157** (31mg, 0.117mmol) and toluenesulfonic acid (catalytic quantity) in water (8ml), petrol (5ml) and methanol (1.5ml) were stirred for ≈48h at room temperature. TLC analysis indicated no starting material was present. Thus the reaction mixture was quenched with solid sodium hydrogen carbonate. Petrol (5ml) and water (5ml) were added, shaken and the aqueous layer removed; the organic layer was then re-extracted with water (2 x 5ml). The combined aqueous fractions were washed with petrol (10ml), the latter being added to the collated organic extracts. The aqueous portion was evaporated *in vacuo* to yield a solid white lump (730mg).

The solid was purified by column chromatography using ethyl acetate : petrol (65:35) to elute the clear and colourless liquid **(164)** (10mg, 0.0526mmol, 45%)[75], a mixture of two isomers in a ratio of 73:27, (determined by ^{1}H NMR spectra integral values for the methoxy group); R_f = 0.37 and 0.40 (100% ethyl acetate). Major isomer, δ_H 0.99 (3H, t, *J* 7, 8-H_3), 1.47, 1.69 and 2.07 (5H, m, 3-H_2, 5-H, 7-H_2), 3.30 (3H, s, OCH_3), 3.41 (1H, m, 5-C<u>H</u>H), 3.64 (1H, dd, *J* 10.5 and 7, 5-CH<u>H</u>), 3.89 (1H, m, 6-H), 4.09 (1H, td, *J* 10.5 and 5, 4-H) and 4.80 (1H, ap d, *J* 3, 2-H); minor isomer, δ_H 1.01 (3H, t, *J* 7, 8-H_3), 1.47, 1.69 and 2.19 (5H, m, 3-H_2, 5-H and 7-H_2), 3.41 (1H, m, 5-C<u>H</u>H), 3.49 (3H, s, OCH_3), 3.64 (1H, dd, *J* 10.5 and 7, 5-CH<u>H</u>), 3.89 (1H, dd, *J* 10.5 and 3.5, 6-H), 4.09 (1H, td, *J* 10.5 and 5, 4-H) and 4.30 (1H, dd, *J* 9.5 and 2, 4-H). *m/z* (CI) (M^+ absent), 159 $[MH\text{-}MeOH]^+$, 84%), 141 (100), 123 (35), 115 (67), 97 (56), 95 (46), 83 (75), 69 (31), 59 (50) and 57 (41).

Concurrent production of (±)-6α-ethyl-4α-hydroxy-5β-hydroxymethyl-2ζ-methoxy-1-oxacyclohexane (164) and (±)-6α-ethyl-4α-hydroxy-5β-hydroxymethyl-1-oxacyclohexan-2-one (165)



(156) **(157)** **(165)** **(164)**

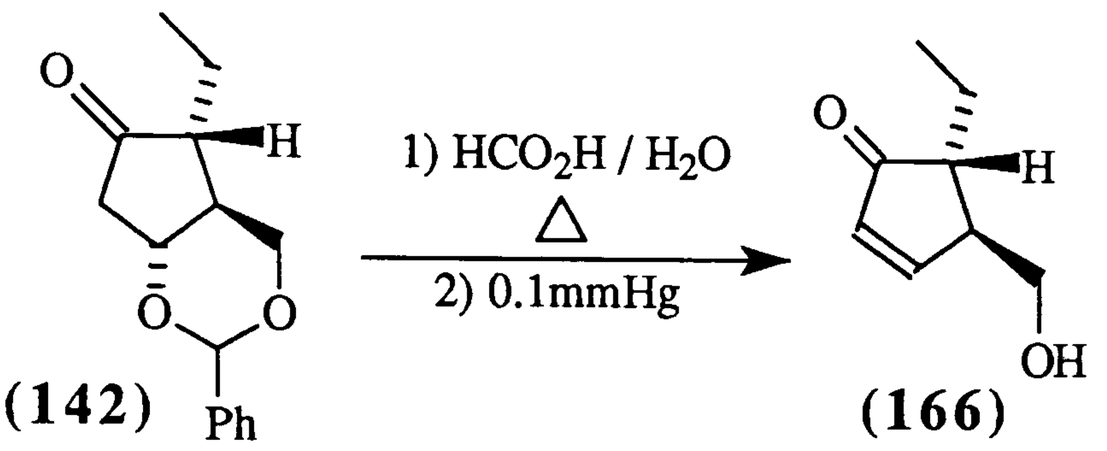
A mixture of the lactone **(156)** and lactol **(157)**, (173mg, approximately 3:5 respectively, as determined by ^{1}H NMR spectroscopy; ≈65mg, ≈0.248mmol **(156)** and ≈108mg, ≈0.409mmol **(157)**) was suspended in anhydrous methanol (12ml) under a nitrogen atmosphere, and CSA (catalytic quantity) was added.

After 24h the R_f had changed from 0.70 to 0.15 and 0.20 (ethyl acetate : petrol, 50:50). Hence the reaction mixture was extracted as usual, but an attempt to dry the organic liquor using anhydrous magnesium sulfate failed as the desiccating agent formed a dense, glutinous cake with the products; to remove any compound adsorbed onto the magnesium sulfate, hot ethyl acetate was used to rinse the magnesium sulfate with filtering. The ethyl acetate was evaporated *in vacuo* to yield the crude extract (154mg). The product was purified by column chromatography eluting with ethyl acetate : petrol (50:50), to give the methoxy acetal **(164)** (67mg, 0.353mmol, 86%), spectral data as previously reported.

Further elution with 100% ethyl acetate produced the dihydroxy lactone **(165)** a pale grey gel, (34mg, 0.196mmol, 79%); R_f = 0.22 (100% ethyl acetate); (Found $[MH]^+$, 175.0975. $C_8H_{15}O_4$ requires MH, 175.0970); υ_{max}/cm^{-1} 1748 (C=O) and 3449 (OH); δ_H 1.04 (3H, t, *J* 7.5, 8-H_3), 1.65 (1H, ap quintet, *J* 7.5, 7-H), 1.82 (2H, m, 5-H, 7-H), 2.55 (1H, dd, *J* 17 and 7, 3β-H), 2.91 (1H, dd, *J* 17 and 5.5, 3α-H), 3.70 (1H, dd, *J* 11 and 6, 5-C<u>H</u>H), 3.95 (1H, dd, *J* 11 and 3.5, 5-CH<u>H</u>), 4.11 (1H, ddd, *J* 10.5, 7.5 and 3, 6-H) and 4.27 (1H, ddd, 9.5, 7 and 5.5, 4-H); δ_C 8.94 (C-8), 26.16 (C-7), 38.88 (C-3), 47.23 (C-5), 61.11 (5-CH_2), 66.72 (C-4), 79.53 (C-6) and 171.64 (C-2); *m/z* (CI) 157

([MH]$^+$, 54%), 145 (8), 140 (10), 139 (100), 127 (49), 121 (20), 115 (40), 113 (30), 111 (30), 109 (12) and 97 (71).

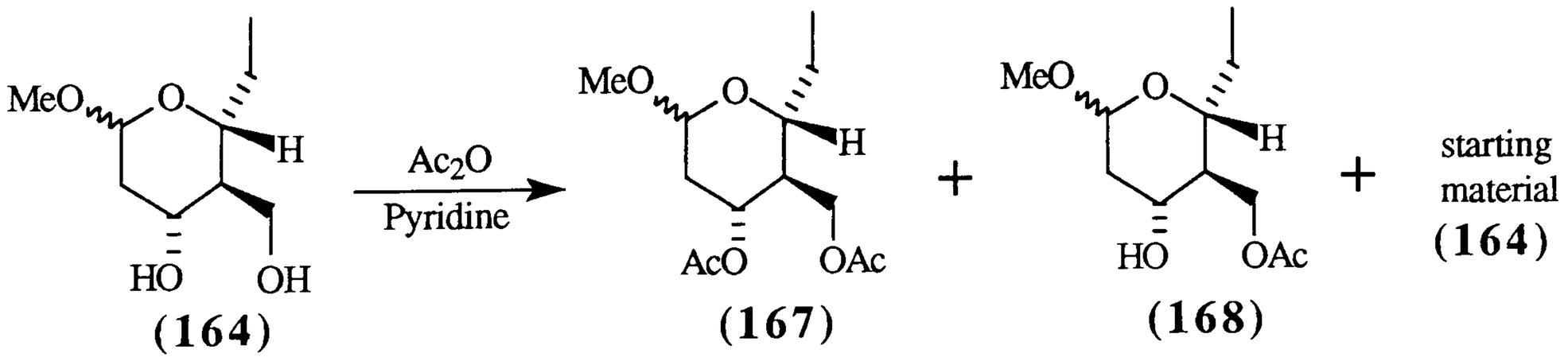
(±)-5α-Ethyl-4β-hydroxymethyl-cyclopent-2-en-1-one (166)



(142) **(166)**

Ketone **(142)** (23mg, 0.0935mmol) was added to formic acid and water (≈1:3, 10ml), and stirred at 50°C for 0.75h. The solvents and benzaldehyde were evaporated *in vacuo* by sequentially rotary evaporator and high vacuum mechanical pump. The crude product was purified by column chromatography eluting with ethyl acetate : petrol (50:50), yielding the clear liquid cylcopentenone **(166)**, (6mg, 46%)[75]; R_f = 0.39 (ethyl acetate : petrol, 65:35); δ_H: 0.94 (3H, t, *J* 7.5, 7-H_3), 1.56 and 1.81 (2H, m, 6-H_2), 2.04 and 2.84 (2H, m, 4-H, 5-H), 3.68 (1H, dd, *J* 10.5 and 6.5, 4-C<u>H</u>H), 3.83 (1H, dd, *J* 10.5 and 5.5, 4-CH<u>H</u>), 6.22 (1H, dd, *J* 5.5 and 2, 2-H) and 7.68 (1H, dd, *J* 5.5 and 2.5, 3-H); *m/z* 140 (M$^+$, 3%), 123 (23), 122 (62), 107 (17), 94 (31) and 79 (100).

Treatment of the methoxy acetal diol (164) with acetic anhydride



(164) **(167)** **(168)**

The diol **(164)** (17mg, 0.0895mmol) in THF (8ml) was added to Ac_2O (dried and distilled from calcium hydride, 0.1ml, 1.06mmol) and anhydrous pyridine (0.3ml) at 0°C, and allowed to reach room temperature. Reaction rate appeared slow by TLC analysis, thus the reaction was not terminated with the standard extraction procedure until after

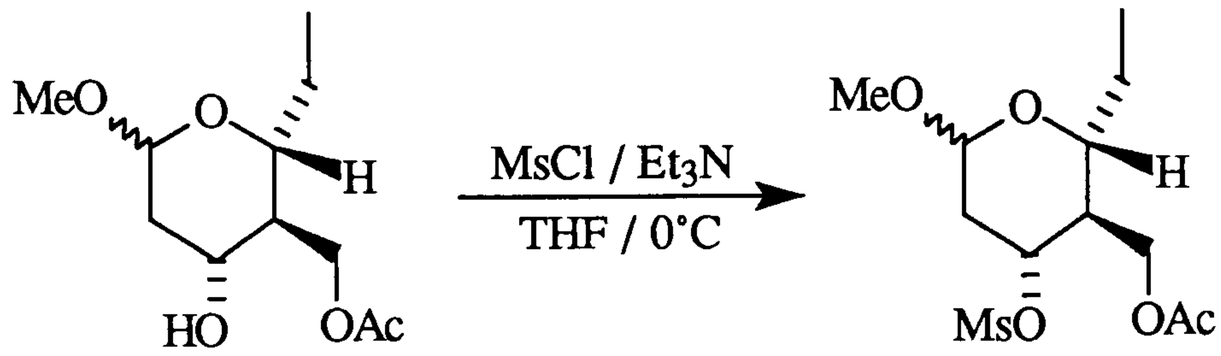
91h.stirring; which yielded a crude product (22mg). The product was purified by column chromatography, eluting with ethyl acetate : petrol (20:80), to give the di-acetate (**167**) (2mg, 0.00730mmol, 8%) as a colourless film, in a ratio of 72:28 of the two isomers (determined by ^{1}H NMR spectra); R_f = 0.70 (ethyl acetate : petrol, 65:35); (M^+ not found); υ_{max}/cm^{-1} 1021 (O-CH_3) and 1739 (C=O). Major isomer, δ_H 1.00 (3H, t, *J* 7.5, 8-H_3), 1.50 and 1.74 (4H, m, 3β-H, 5-H, 7-H_2), 2.04 and 2.06 (each 3H, each s, each O_2CCH_3), 2.22 (1H, ddd, *J* 12.5, 5 and 1.5, 3α-H), 3.33 (3H, s, OCH_3), 3.69 (1H, m, 6-H), 4.04 (1H, dd, *J* 12 and 2.5, 5-C<u>H</u>H), 4.17 (1H, dd, *J* 12 and 3, 5-CH<u>H</u>), 4.81 (1H, dd, *J* 3.5 and 1.5, 2-H) and 5.19 (1H, td, *J* 12.5 and 5, 4-H); δ_C 9.40 (C-8), 20.02 and 21.08 (2 x $O_2C\underline{C}H_3$), 25.18 (C-7), 35.70 (C-3), 44.41 (C-5), 54.56 (OCH_3), 60.15 (5-CH_2), 66.77 (C-6), 69.15 (C-4), 98.02 (C-2) and 170.26 and 171.10 (2 x $O_2\underline{C}CH_3$). Minor isomer, δ_H 1.03 (3H, t, *J* 7.5, 8-H_3), 1.50 and 1.74 (4H, m, 3β-H, 5-H, 7-H_2), 2.04 and 2.05 (each 3H, each s, each O_2CCH_3), 2.33 (1H, ddd, *J* 12, 4.5 and 2, 3α-H), 3.29^ (1H, m, 6-H), 3.51 (3H, s, OCH_3), 4.04 (1H, dd, *J* 12 and 2.5, 5-C<u>H</u>H), 4.20 (1H, dd, *J* 12 and 3, 5-CH<u>H</u>), 4.37 (1H, dd, *J* 10 and 2, 2-H) and 4.96 (1H, td, *J* 12.5 and 5, 4-H); δ_C 9.48 (C-8), 20.80 and 21.02 (2 x $O_2C\underline{C}H_3$), 25.24 (C-7), 37.08 (C-3), 44.49 (C-5), 54.51 (OCH_3), 60.32 (5-CH_2), 66.83 (C-6), 68.45 (C-4), 98.10 (C-2) and 170.26 and 171.92 (2 x $O_2\underline{C}CH_3$); (*Signals overlap with water signal; ^ signal partially obscured by methoxy singlet of major isomer). *m/z* (FAB) 297 (100%), 274 (M^+, 3%), 243 (15), 214 (4), 183 (39), 157 (7), 154 (4), 147 (6), 123 (67) and 113 (6).

Further elution with ethyl acetate : petrol (40:60) gave the mono-ester (**168**) (10mg, 0.0431mmol, 48%) also as a colourless film, in a ratio of 78:22 of isomers (determined by ^{1}H NMR spectra); R_f = 0.43 (ethyl acetate : petrol, 65:35); (M^+ not found); υ_{max}/cm^{-1} * 1736 (C=O) and 3436 (OH). Major isomer, δ_H 1.00 (3H, t, *J* 7.5, 8-H_3), 1.49, 1.65 and 1.79 (4H, m, 3β-H, 5-H, 7-H_2), 2.10 (3H, s, O_2CCH_3), 2.13^ (1H, m, 3α-H), 2.73 (1H, br d, *J* 3.5, OH), 3.32 (3H, s, OCH_3), 3.56 (1H, m, 6-H), 3.85 (1H, m, 4-H), 4.05 (1H, dd, *J* 12 and 3, 5-C<u>H</u>H), 4.62 (1H, dd, *J* 12 and 3, 5-CH<u>H</u>) and 4.81 (1H, d, *J* 3.5, 2-H) (^ peaks partly obscured by methyl singlet); δ_C 9.56 (C-8), 20.95 ($O_2C\underline{C}H_3$), 25.18 (C-7), 38.40 (C-3), 48.30 (C-5), 54.57 (OCH_3), 61.01 (5-CH_2), 63.84 (C-6), 69.13 (C-4), 98.56 (C-2) and 171.98 ($O_2\underline{C}CH_3$). Minor isomer, δ_H 1.03 (3H, t, *J* 7.5, 8-H_3), 1.49, 1.65 and 1.79 (4H, m,

3β-H, 5-H, 7-H_2), 2.09 (3H, s, O_2CCH_3), 2.23 (1H, ddd, *J* 12, 5 and 1.5, 3α-H), 2.73 (1H, br d, *J* 3.5, OH), 3.18 (1H, td, *J* 9.5 and 2.5, 6-H), 3.51 (3H, s, OCH_3), 3.85 (1H, m, 4-H), 4.07 (1H, dd, *J* 12 and 3, 5-C<u>H</u>H), 4.32 (1H, dd, *J* 10 and 2, 2-H) and 4.59 (1H, dd, *J* 12 and 3, 5-CH<u>H</u>); δ_C 9.76 (C-8), 20.87 ($O_2C\underline{C}H_3$), 25.44 (C-7), 39.92 (C-3), 48.10 (C-5), 56.27 (OCH_3), 60.88 (5-CH_2), 66.86 (C-6), 73.97 (C-4), 100.74 (C-2) and 171.77 ($O_2\underline{C}CH_3$). *m/z* (CI) 201 ($[MH\text{-}MeOH]^+$, 4%), 197 (2), 185 (2), 154 (33), 123 (21), 85 (61) and 83 (100); *m/z* (FAB) 255 (100%), 233 ($[MH]^+$, 2), 231 ($[M\text{-}H]^+$, 6), 201 ($[M\text{-}MeO]^+$, 59), 147 (4), 113 (8), 112 (12), 111 (53), 110 (9) and 109 (91).

Further elution with 100% ethyl acetate gave the starting diol **(164)** (5mg, 0.0263mmol, 29%), a beige semi-solid.

(±)-5β-Acetoxymethyl-6α-ethyl-2ζ-methoxy-4α-methyloxysulfonyl-1-oxacyclohexane (170)

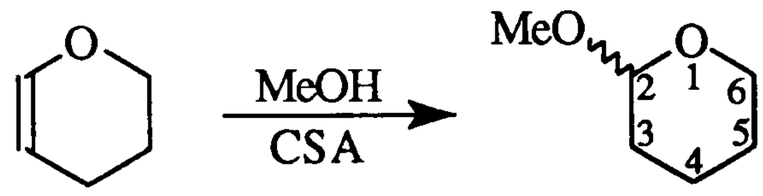


(168) **(170)**

The mono-acetate **(168)** (10mg, 0.0431mmol) was dissolved at 0°C in THF with triethylamine (0.5ml, 3.59mmol) and methanesulfonyl chloride (0.1ml, 1.29mmol). After 1.25h no starting material was apparent by TLC, and thus the reaction was worked up as usual, except using ether as solvent. Purification by column chromatography (ethyl acetate : petrol, 20:80) gave the mesylate **(170)** as a very pale brown liquid, (10mg, 0.0323mmol, 75%), in a ratio of the two isomers of 82:18 (determined by ^1H NMR spectra); R_f = 0.71 (ethyl acetate : petrol, 65:35); (M^+ not found); υ_{max}/cm^{-1} 799 (C-O-S), 1092 and 1352 (S=O) and 1740 (C=O). Major isomer, δ_H 1.00 (3H, t, *J* 7, 8-H_3), 1.51 (1H, dq, *J* 8 and 7, 7-<u>H</u>H), 1.76 (2H, m, 5-H, 7-H<u>H</u>), 1.93 (1H, ddd, *J* 12.5, 11.5 and 4, 3β-H), 2.09 (3H, s, O_2CCH_3), 2.44 (1H, ddd, *J* 12.5, 5 and 1, 3α-H), 3.01 (3H, s, O_3SCH_3), 3.32 (3H, s, OCH_3), 3.70 (1H, ddd, *J* 10.5, 8 and 3, 6-H), 4.15 (1H, dd, *J* 12 and 2.5, 5-C<u>H</u>H), 4.27 (1H, dd, *J* 12 and 3, 5-CH<u>H</u>), 4.83 (1H, ap d, *J* 3, 2-H) and 5.09 (1H, td, *J* 11.5 and 5, 4-H); δ_C

9.40 (C-8), 20.83 ($O_2C\underline{C}H_3$), 25.14 (C-7), 37.43 (C-3), 38.38 (C-5), 48.96 (O_3SCH_3), 54.67 (OCH_3), 59.57 (5-CH_2), 68.99 (C-6), 74.83 (C-4), 97.88 (C-2) and 170.90 ($O_2\underline{C}CH_3$). Minor isomer: δ_H 1.00 (3H, t, *J* 7, 8-H_3), 1.47 and 1.76 (3H, m, 5-H, 7-H_2), 1.86 (1H, m, 3β-H), 2.08 (3H, s, O_2CCH_3), 2.54 (1H, ddd, *J* 12.5, 5 and 2, 3α-H), 3.04 (3H, s, O_3SCH_3), 3.27 (1H, m, 6-H), 3.51 (3H, s, OCH_3), 4.27 (1H, dd, *J* 12 and 3, 5-C$\underline{H}$H), 4.29 (1H, dd, *J* 12 and 3, 5-CH$\underline{H}$), 4.35 (1H, dd, *J* 9.5 and 2, 2-H) and 4.87 (1H, td, *J* 11 and 5, 4-H); δ_C 9.54 (C-8), 20.75 ($O_2C\underline{C}H_3$), 25.23 (C-7), 37.45 (C-3), 39.28 (C-5), 44.90 (O_3SCH_3), 54.67 (OCH_3), 59.57 (5-CH_2), 68.81 (C-6), 76.81 (C-4), 98.12 (C-2) and 170.90 ($O_2\underline{C}CH_3$). *m/z* (CI) 279 ([MH-MeOH]$^+$, 66%), 237 (6), 219 (14), 184 (14), 183 (79), 157 (27), 155 (65), 141 (70), 143 (57) and 97 (100).

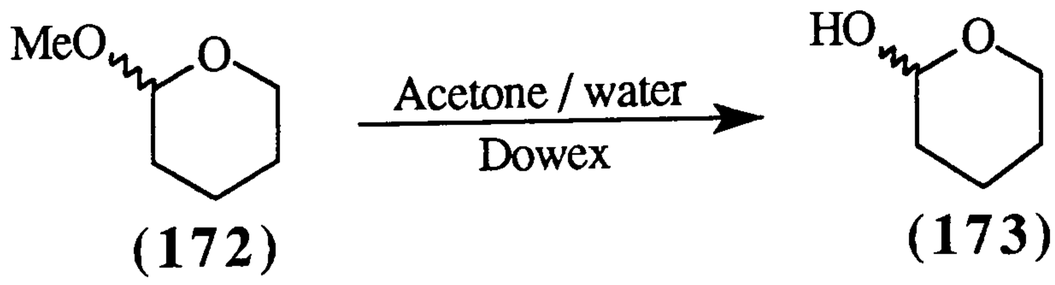
2ξ-Methoxy-1-oxacyclohexane (172)



(172)

3,4-dihydro-2*H*-pyran (420mg, 5.00mmol) was dissolved in methanol (5ml) and CSA (27mg) and stirred for 1.25h. Then, as the starting material had disappeared by TLC, the reaction underwent the standard work-up. This returned the clear liquid **(172)** (522mg, 4.50mmol, 90%)[89]; R_f = 0.75 (ethyl acetate : petrol, 30:70); δ_H (300 MHz) 1.55 and 1.79 (6H, m, 3-H_2, 4-H_2, 5-H_2), 3.32 (3H, s, OCH_3), 3.61 and 3.82 (2H, m, 6-H_2) and 4.38 (1H, t, *J* 5.5, 2-H); *m/z* (CI) 101 (16%), 97 (13), 87 (11), 85 ([MH-MeOH]$^+$, 100), 83 (97), 71 (18), 69 (11), 61 (35), 59 (13) and 57 (33).

2ξ-Hydroxy-1-oxcyclohexane (173)

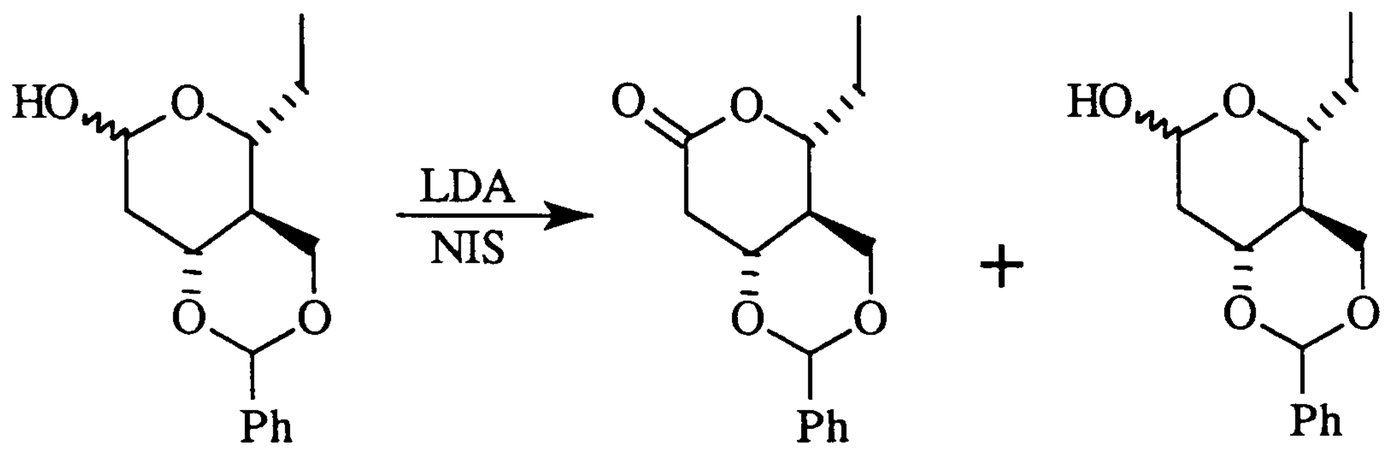


(172) **(173)**

The methoxy acetal **(172)** (522mg, 4.5mmol) was dissolved in acetone (3ml), water (2ml) and acidic Dowex© (11mg) added and stirred at room temperature. After 0.5h, all

starting material had disappeared by TLC, so the usual extraction procedure was followed. Following column chromatography with ethyl acetate : petrol (20:80), a colourless and transparent liquid (**173**) was obtained (394mg, 3.86mmol, 86%)[122]; R_f = 0.54 (ethyl acetate : petrol, 30:70); δ_H 1.59 (4H, m, 4-H_2 and 5-H_2), 1.77 and 1.84 (2H, m, 3-H_2), 3.51 and and 3.88 (2H, m, 6-H_2) and 4.96 (1H, t, *J* 5, 2-H); *m/z* (CI) 103 ($[MH]^+$, 4%), 101 (11), 93 (11), 85 (77), 83 (100), 81 (3), 79 (32), 71 (11), 69 (8) and 57 (28).

Reaction of δ-Lactol (157) with NIS and LDA



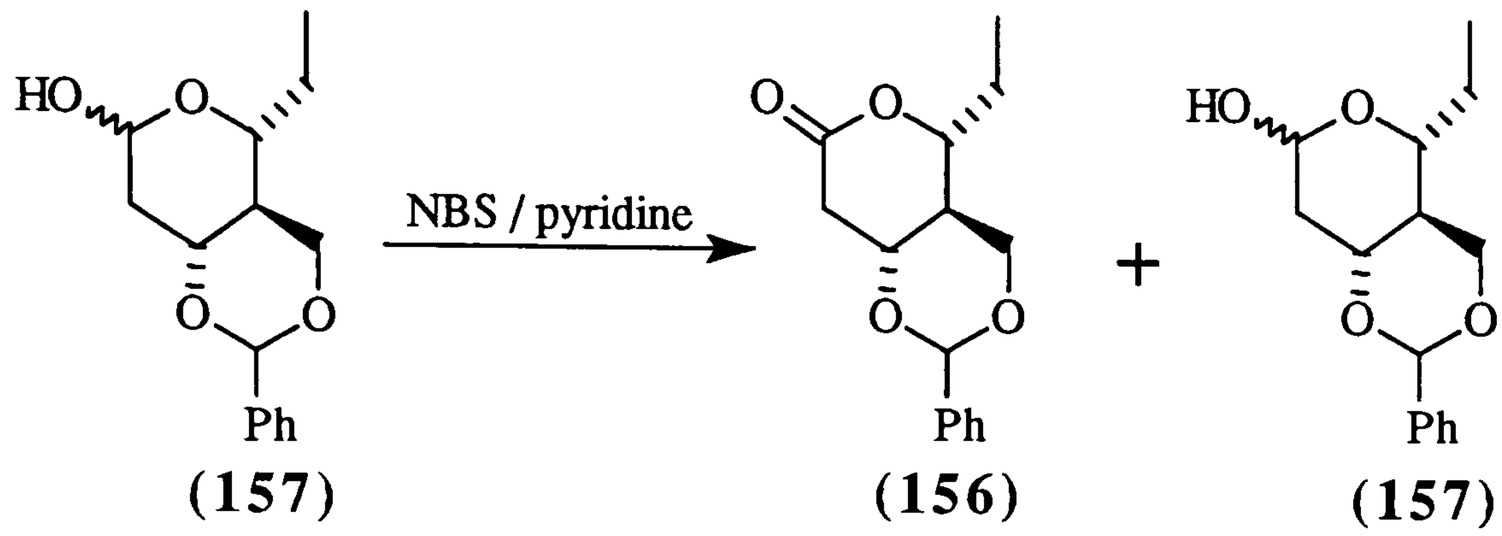
(157) **(156)** **(157)**

n-Butyl lithium (0.22ml of a 2.5M solution in hexanes, 0.55mmol) was added to diisopropylamine (0.04ml, 0.283mmol) in THF (6ml) at -78°C under nitrogen and stirred for 0.25h with warming to ≈10°C, to prepare a solution of LDA. The temperature was lowered to -78°C, then the δ-valerolactol (**157**) (66mg, 0.25mmol) in THF (3ml) was added; the mixture was allowed to warm to 15°C over 0.5h, with no colour change seen in the solution. The reaction mixture was again lowered to -78°C and N-iodosuccinimide (65mg, 0.289mmol) added, the reaction mixture retaining the pale orange colour of the N-iodosuccinimide. No change in R_f was detected after 1h, thus the reaction mixture was held at room temperature for 2.5h.

However no change was seen still by TLC from starting material, thus dilute aqueous hydrochloric acid was carefully added until *p*H9 was attained. Extraction produced a bright orange solid (160mg), which was several spots by TLC analysis. The crude mixture was purified by column chromatography, eluting with ethyl acetate : petrol (30:70) yielding a pale yellow solid (11mg, ≈0.042mmol, ≈17%). The product was a mixture of starting material (**157**) and protected δ-lactone (**156**), which

corresponded with previous data[75]; ^{1}H NMR spectroscopy determined a ratio of 1:1.3 respectively. None of the other products could be identified.

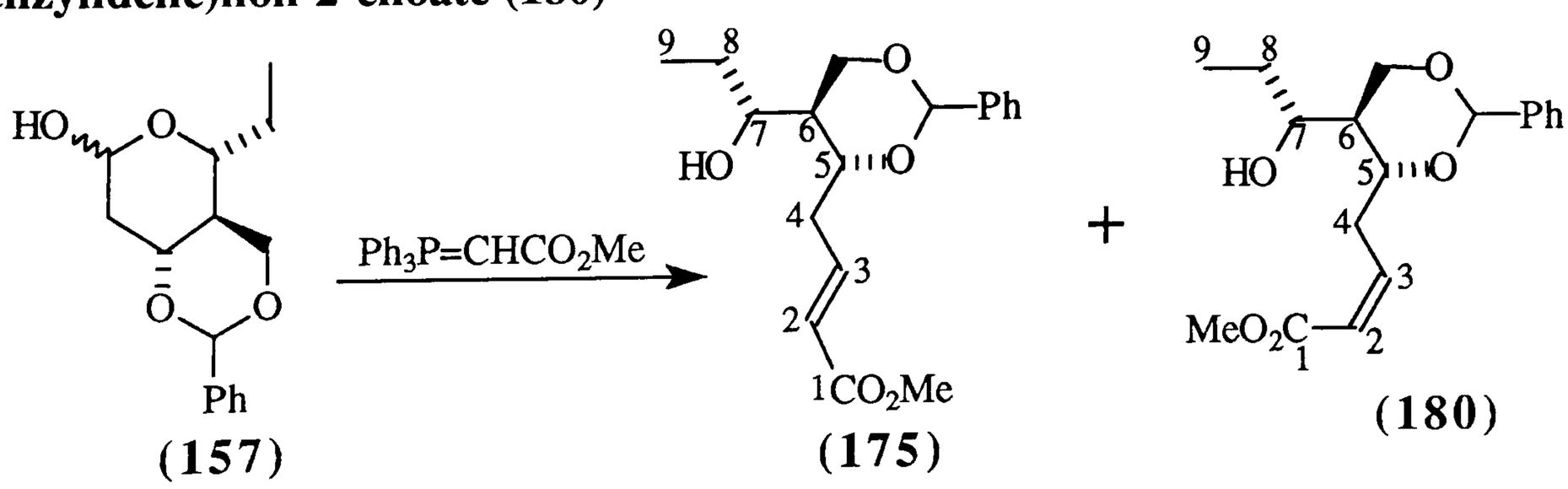
Reaction of δ-Lactol (157) with NBS and Pyridine



(157) **(156)** **(157)**

Under anhydrous conditions, pyridine (0.1ml, 1.24mmol) was added to the lactol **(157)** (26.4mg, 0.10mmol) dissolved in THF (4ml) and stirred for 0.2h. The temperature was then lowered to 0°C, and NBS (21mg, 0.128mmol) added. After ≈100h stirring no change in R_f value from the starting material **(157)** was detected, so the standard extraction was made to give a pale yellow solid (41mg, >100% possible yield). The pure products were obtained by column chromatography, eluting with ethyl acetate : petrol (30:70) yielding a mixture of a white solid and clear liquid of $R_f = 0.37$ (17mg, ≈0.065mmol, ≈65%), itself a mixture of the starting lactol **(157)** and lactone **(156)**; in a ratio of lactol to lactone of 5.1:1 by ^{1}H NMR spectroscopy.

(±)-Methyl (2*E*,5*R,6*R**,7*R**)-7-hydroxy-5-O,6-(methyl-O)-(benzylidene)non-2-enoate (175) and (±)-methyl (2*Z*,5*R**,6*R**,7*R**)-7-hydroxy-5-O,6-(methyl-O)-(benzylidene)non-2-enoate (180)**

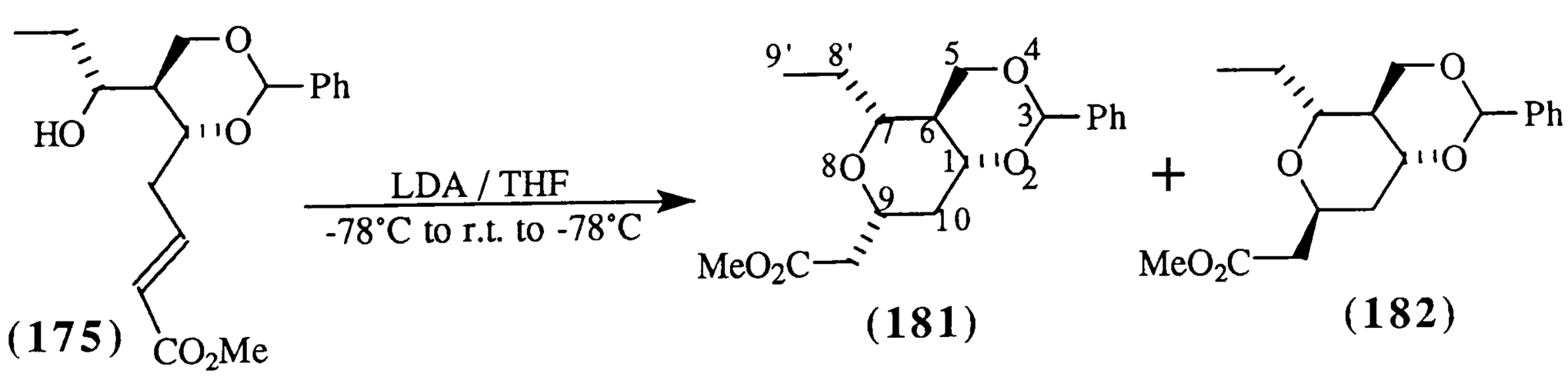


(157) **(175)** **(180)**

The lactol **(157)** (84mg, 0.318mmol) and methyl (triphenyl-phosphoranylidene) acetate (789mg, 2.36mmol) in acetonitrile (40ml) were heated to reflux for 19h. Upon cooling, the solvent was evaporated and the product was purified by flash chromatography. Elution with ethyl acetate : petrol (20:80), gave the *Z*-unsaturated ester **(180)** (18mg, 0.0563mmol, 18%) as a colourless film; R_f = 0.49 (ethyl acetate : petrol, 40:60)[75]; δ_H (300 MHz) 1.01 (3H, t, *J* 7.5, 9-H_3), 1.52 and 2.05 (3H, m, 6-H, 8-H_2), 2.94 and 3.33 (2H, m, 4-H_2), 3.53 (1H, m, 7-H), 3.72 (3H, s, CO_2CH_3), 3.78 (1H, t, *J* 11.5, 6-$CH_2\beta$), 3.97 (1H, m, 5-H), 4.24 (1H, dd, *J* 11.5 and 5, 6-$CH_2\alpha$), 5.45 (1H, s, O-CH-O), 5.91 (1H, ap dt, *J* 11.5 and 1.5, 2-H), 6.59 (1H, dt, *J* 11.5 and 7.5, 3-H), 7.36 (3H, m, Ar-H_3) and 7.47 (2H, m, Ar-H_2); *m/z* (CI) 321 ($[MH]^+$, 100%), 319 (21), 303 (8), 221 (14), 215 (76) and 197 (59).

Further elution with ethyl acetate : petrol (25:75) gave the *E*-unsaturated ester **(175)**, (70mg, 0.219mmol, 69%) also as a colourless film; R_f = 0.42 (ethyl acetate : petrol, 40:60)[75]; δ_H (300 MHz) 0.99 (3H, t, *J* 7.5, 9-H_3), 1.48 and 2.04 (3H, m, 6-H, 8-H_2), 2.61 and 2.84 (2H, m, 4-H_2), 3.51 (1H, m, 7-H), 3.73 (3H, s, CO_2CH_3), 3.74 (1H, t, *J* 11.5, 6-$CH_2\beta$), 3.93 (1H, m, 5-H), 4.25 (1H, dd, *J* 115 and 5, 6-$CH_2\alpha$), 5.45 (1H, s, O-CH-O), 5.94 (1H, ap.dt, *J* 16 and 2.5, 2-H), 7.13 (1H, ddd, *J* 16, 7.5 and 7, 3-H), 7.35 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2); *m/z* (CI) 321 (M^+, 50%), 320 (7), 319 (26), 289 (15), 221 (42), 215 (45), 165 (69) and 107 (100).

Treatment of the unsaturated ester (175) with LDA



(175) **(181)** **(182)**

n-Butyl lithium (0.05ml of a 2.5M solution in hexanes, 0.125mmol) was added to diisopropylamine (0.02ml, 0.142mmol) in THF (20ml) at -78°C under nitrogen; the mixture was allowed to warm to room temperature and stirred for 0.5h to prepare a

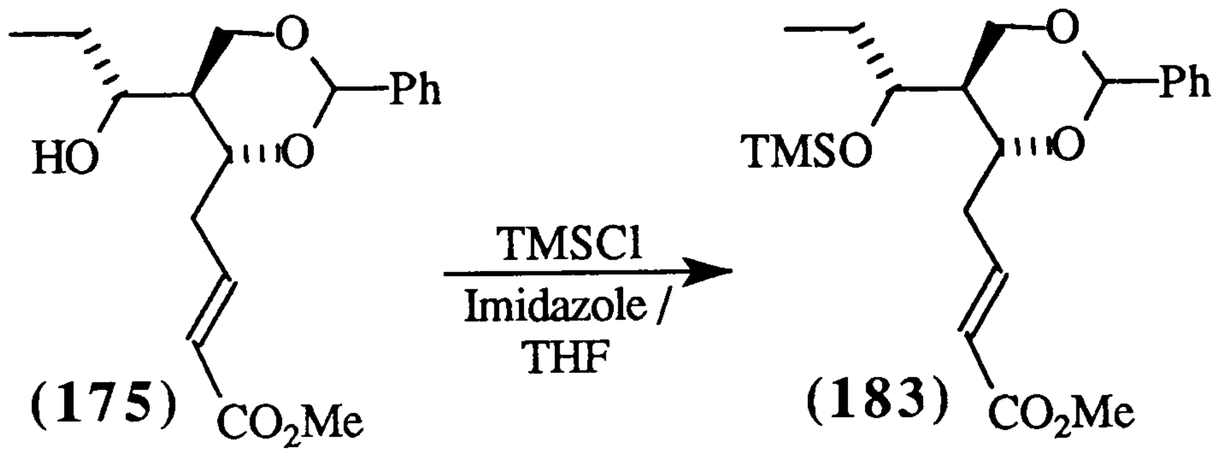
solution of lithium diisopropylamide. The temperature was then lowered to -78°C. The *E*-unsaturated ester **(175)** (36mg, 0.113mmol) in THF (20ml) was added to the solution of LDA over 15 minutes. This was then allowed to reach room temperature over 2.25h, then recooled to -78°C. Half of the volume was transferred to another flask containing water, and to the remainder was added deuterium oxide. The protic quenching system was diluted with more water (4ml), and extracted as normal to yield a gum (70mg). The product was purified by column chromatography, elution with ethyl acetate : petrol (50:50), gave the 9α-ethyl methylcarboxylate bicyclo ester **(181)** (14mg, 0.0438mmol, 78%) as a colourless film; R_f = 0.64 (ethyl acetate : petrol, 30:70); (Found M^+, 320.1616. $C_{18}H_{24}O_5$ requires M, 320.1624); υ_{max}/cm^{-1} * 1733 (C=O); δ_H (300 MHz) 0.94 (3H, t, *J* 7.5, 9'-H_3), 1.46 (2H, m, 8'-H_2), 1.57 (1H, m, 10α-H), 1.79 (1H, dtd, *J* , 14, 10 and 4.5, 6-H), 2.03 (1H, ddd, *J* 12, 4.5 and 2, 10β-H), 2.48 (1H, dd, *J* 15 and 5.5, 9-C<u>H</u>H), 2.64 (1H, dd, *J* 15 and 8, 9-CH<u>H</u>), 3.07 (1H, ddd, *J* 10, 8.5 and 3, 7-H), 3.62 (1H, t, *J* 11, 5β-H), 3.69 (3H, s, CO_2CH_3), 3.81 (1H, ddd, *J* 14, 10 and 4.5, 1-H), 3.96 (1H, dddd, *J* 12, 8, 5.5 and 2, 9-H), 4.17 (1H, dd, *J* 11 and 4.5, 5α-H), 5.58 (1H, s, 3-H), 7.35 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2); δ_C 9.43 (C-9'), 25.34 (C-8'), 36.89 and 41.02 (C-10, 9-CH_2), 44.10 (C-6), 51.69 ($CO_2\underline{C}H_3$), 67.88 (C-5), 72.48 and 76.58 (C-7, C-9), 79.02 (C-1), 101.66 (C-3), 126.13, 128.36, 128.99 and 138.10 (aromatics) and 171.50 ($\underline{C}O_2CH_3$); *m/z* 320 (M^+, 92%), 319 (88), 289 (13), 262 (20), 261 (48), 247 (10), 243 (16), 217 (14), 140 (55) and 105 (100).

Further elution with ethyl acetate : petrol (20:80) produced as a colourless film a mixture of the two diastereomers (3mg) which is 72% 9β-$CH_2CO_2CH_3$ isomer **(182)** (≡2.1mg, 0.00656mmol, 12%) and 28% the main product **(181)** (≡0.9mg, 0.00281mmol, 5%), by ^1H NMR spectra integral analysis. The data for **(182)** was: R_f = 0.48 (ethyl acetate : petrol, 30:70); (Found M^+, 320.1610. $C_{18}H_{24}O_5$ requires M, 320.1624); υ_{max}/cm^{-1} * 1743 (C=O); δ_H (300 MHz) 0.91 (3H, t, *J* 7.5, 9'-H_3), 1.44 (2H, m, 8'-H_2), 1.79 (1H, m, 6-H), 1.90 (1H, ddd, *J* 13, 5 and 1.5, 10α-H), 2.06 (1H, ddd, *J* 13, 6 and 1.5, 10β-H), 2.54 (1H, dd, *J* 14.5 and 5.5, 9-C<u>H</u>H), 2.92 (1H, dd, *J* 14.5 and 10, 9-CH<u>H</u>), 3.33 (1H, ddd, *J* 11, 8.5 and 3, 7-H), 3.64 (1H, t, *J* 11, 5β-H), 3.71 (3H, s, CO_2CH_3), 3.94 (1H, m, 1-H), 4.18 (1H, dd, *J* 5.5 and 11, 5α-H), 4.66 (1H, dddd, *J* 10,

6, 5.5 and 1.5, 9-H), 5.59 (1H, s, 3-H), 7.36 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2); δ_C (300 MHz) 9.40 (C-9'), 25.42 (C-8'), 33.90 and 37.67 (C-10, 9-CH_2), 44.41 (C-6), 51.82 ($CO_2\underline{C}H_3$), 69.98, 70.99 and 75.36 (C-5, C-7, C-9), 79.01 (C-1), 101.70 (C-3), 126.10, 128.36, 129.03 and 138.21 (aromatics) and 171.27 ($\underline{C}O_2CH_3$); *m/z* 320 (M^+, 48%), 319 (50), 289 (5), 261 (27), 247 (11), 185 (14),140 (29), 129 (33), 105 (73) and 57 (100).

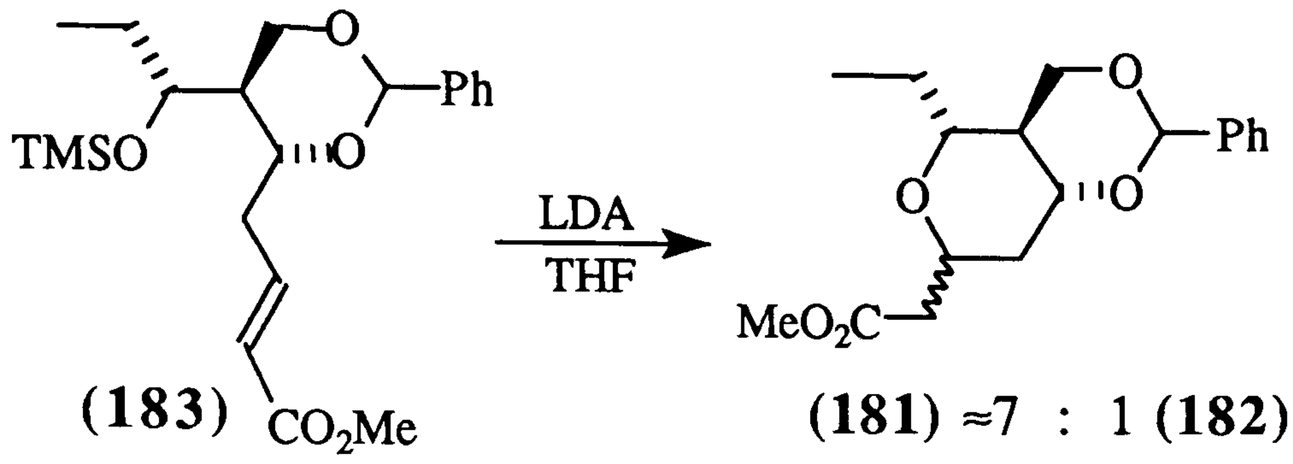
The deuterium oxide quench portion was extracted in a likewise manner, to yield a mixture (31mg), which by ^1H, ^{13}C NMR and MS were identical to products found previously ((**181**) and (**182**)) and in a very similar ratio; no incorporation of deuterium was observed.

(±)-Methyl (2*E*,5*R,6*S**,7*R**)-5-O,6-(methyl-O)-(benzylidene)-7-trimethylsilyloxynon-2-enoate (183)**



To the *E*-unsaturated ester (**175**) (18mg, 0.056mmol) in THF (6ml) was added imidazole (28mg, 0.411mmol) and chlorotrimethylsilane (0.03ml, 0.236mmol), and stirred for 17h. The reaction was extracted as normal, except ether was used. The crude product (**183**) was a clear liquid (39mg, >100% crude product); R_f = 0.81 (ethyl acetate: petrol, 30:70); δ_H 0.13 (6H, s, $Si(CH_3)_2$), 0.15 (3H, s, $SiCH_3$), 0.99 (3H, t, *J* 7.5, 9-H_3), 1.22, 1.40 and 2.05 (3H, m, 6-H, 8-H_2), 2.62 (1H, ddd, *J* 15.5, 7.5 and 1.5, 4-H), 2.85 (1H, m, 4-H), 3.64 (1H, m, 7-H), 3.73 (3H, s, CO_2CH_3), 3.76 (1H, t, *J* 11.5, 6-$CH_2\beta$), 3.91 (1H, m, 5-H), 4.26 (1H, dd, *J* 11.5 and 5, 6-$CH_2\alpha$), 5.46 (1H, s, O-CH-O), 5.94 (1H, dt, *J* 15.5 and 1.5, 2-H), 7.12* (1H, ddd, *J* 15.5, 7.5 and 6.5, 3-H), 7.36 (3H, m, Ar-H_3) and 7.48 (2H, m, Ar-H_2), (* signal obscured by broad singlet at 7.08ppm); *m/z* (CI) 391 ([M-H]$^+$, 2%), 369 (1), 301 (1), 319 (3), 302 (3), 257 (3), 261 (8), 226 (3), 215 (5), 213 (4), 197 (10) and 100 (100).

Treatment of TMS derivative (183) with LDA

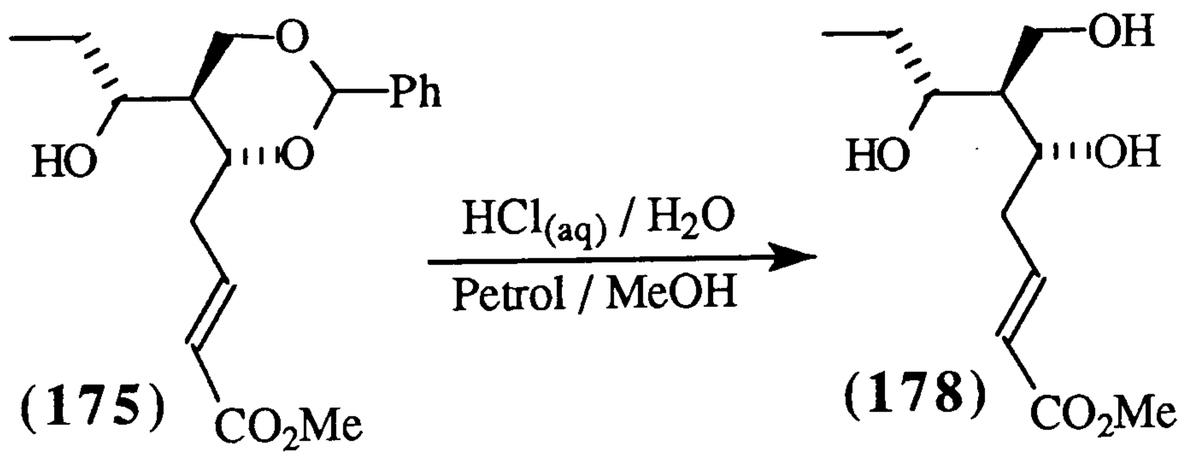


(183) **(181)** ≈7 : 1 **(182)**

The attempted elimination reaction was repeated using the silyl ether **(183)** (22mg, 0.056mmol). The crude oil (14mg, >100% possible yield (9.0mg, 0.0281mmol)) obtained from the aqueous quench was identical by TLC and ^{1}H NMR spectra analysis, and the products in a similar ratio, to the the mixture obtained from the analogous reaction on the alcohol **(175)**.

Upon extracting the the portion reacted with D_2O, the product also had cyclised to the ethers **(181)** and **(182)** (37mg, >100% possible yield (9.0mg, 0.0281mmol)) and again no deuterium could be found in the molecule by either ^{1}H or ^{13}C NMR spectra, nor both low and high resolution EI and CI MS analysis.

(±)-Methyl (2*E*,5*R,6*R**,7*R**)-5,7-dihydroxy-6-hydroxymethylnon-2-enoate (178)**

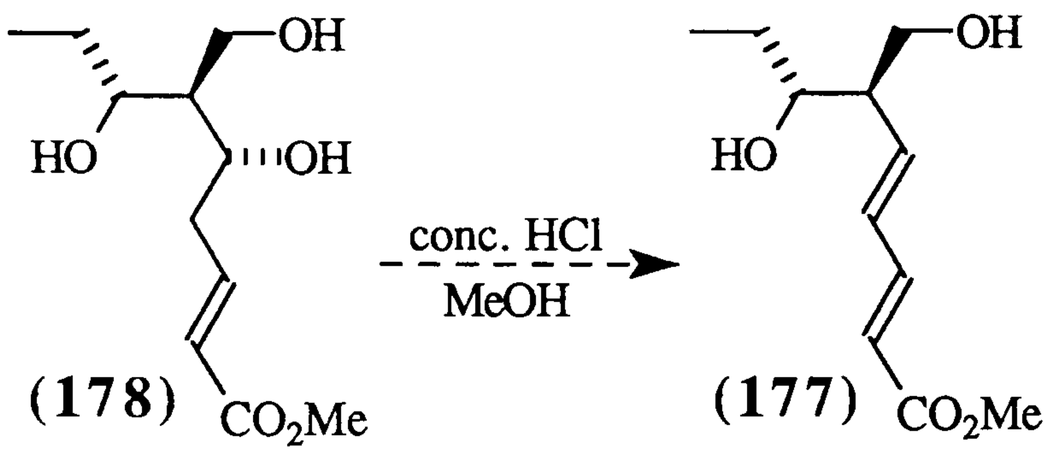


(175) **(178)**

The acetal **(175)** (20mg, 0.0625mmol) in methanol (6ml), petrol (10ml), water (10ml) and 1M hydrochloric acid (1ml) was stirred for 2.25h at room temperature. The reaction system was extracted as normal to yield a brown liquor (12mg). The product was purified by column chromatography, eluting with ethyl acetate, to give the triol ester as a clear liquid, **(178)** (5mg, 0.0216mmol, 34%); R_f = 0.36 (ethyl acetate); (Found $[MH]^+$, 233.1387. $C_{11}H_{21}O_5$ requires MH, 233.1389); δ_H (300 MHz) 0.99 (3H, t, *J* 7.5, 9-H_3), 1.46 and 1.62 (3H, m, 6-H, 8-H_2), 2.43 and 2.64 (2H, m, 4-H_2), 2.82 (1H, br s, OH), 3.41 (1H, br s, OH), 3.74 (3H, s, CO_2CH_3), 3.90 (1H, dd, *J* 11.5 and 3.5, 6-CH<u>H</u>),

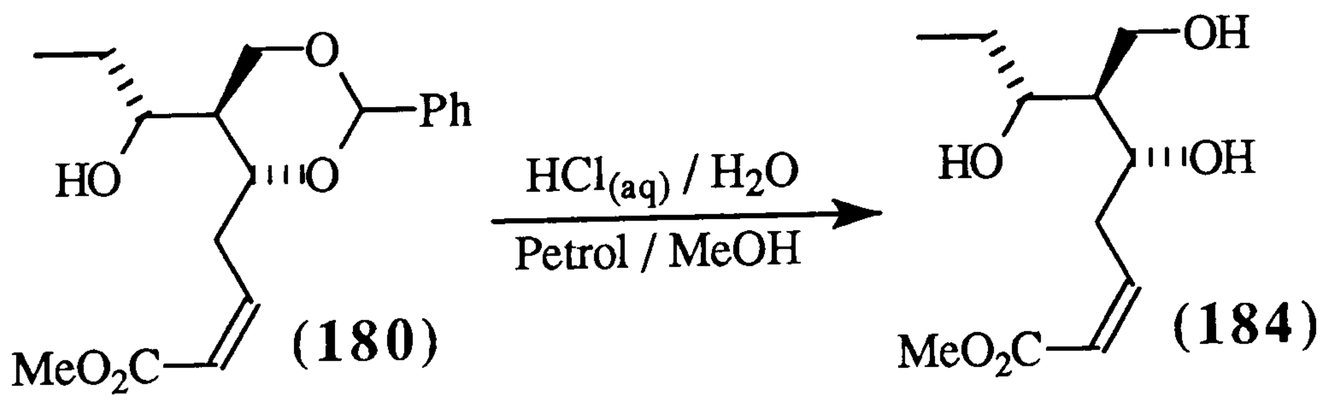
4.00 and 4.33 (2H, m, 5-H, 7-H), 4.14 (1H, dd, *J* 11.5 and 4.5, 6-C<u>H</u>H), 5.94 (1H, ap dt, *J* 15.5 and 1.5, 2-H) and 6.98 (1H, ap dt, *J* 15.5 and 7.5, 3-H); δ_C 10.41 (C-9), 27.97 (C-8), 38.29 (C-4), 46.35 (C-6), 51.59 ($CO_2\underline{C}H_3$), 62.51 (6-CH_2), 70.38 and 75.43 (C-5, C-7), 123.44 (C-2), 145.30 (C-3) and 166.77 (C-1); *m/z* (CI) 233 ($[MH]^+$, 100%), 215 (21), 201 (16), 197 (39), 183 (50), 179 (39), 165 (83), 139 (46) and 115 (45).

(±)-Methyl (2*E*,4*E*,6*R,7*R**)-7-hydroxy-6-hydroxymethylnon-2,4-dienoate (177)**



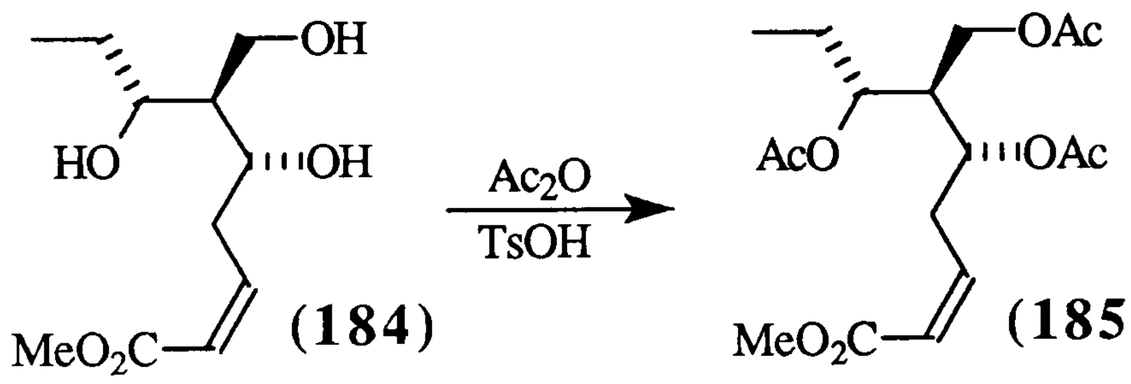
The alcohol **(178)** (5mg, 0.0216mmol) in MeOH (12ml) and 10M hydrochloric acid (2ml) was stirred at room temperature for 2.25h, and as no change in TLC value was noted, the reaction was heated to 39°C for 5.75h; an apparent increase in R_f value was noted (from 0.36 to 0.51, 100% ethyl acetate). The product was extracted and evaporated *in vacuo* to give a clear film (21mg, >100% possible yield); analysis by ^1H NMR spectra appeared to contain starting material only. The CI MS indicated the presence of the desired di-ene **(177)**, (Found $[MH]^+$, 215.1285. $C_{11}H_{19}O_4$ requires MH, 215.1283); *m/z* (CI) 215 ($[MH]^+$, 1%), 197(3), 181 (1), 179 (1), 167 (4), 149 (8), 91 (26), 87 (10), 85 (65) and 83 (100). However, dehydration is a highly favoured process at low pressure, so the eliminated product probably formed in the Autospec®.

(±)-Methyl (2*Z*,5*R,6*R**,7*R**)-5,7-dihydroxy-6-hydroxymethylnon-2-enoate (184)**



The *Z*-alkene **(180)** (18mg, 0.0563mmol) was treated in a similar manner to the *E*-product **(175)** to enable synthesis of the *Z*-triol **(184)**, purification by column chromatography, eluting with ethyl acetate, gave a clear film (9mg, 0.0388mmol, 69%); R_f = 0.36 (ethyl acetate); (Found $[MH]^+$, 233.1387. $C_{11}H_{21}O_5$ requires MH, 233.1389); δ_H 0.99 (3H, t, *J* 7.5, 9-H_3), 1.52, 1.63 and 1.68 (1H, m, 6-H, 8-H_2), 2.95 (2H, m, 4-H_2), 3.61 (1H, br. s, OH), 3.74 (3H, s, CO_2CH_3), 3.92 (1H, dd, *J* 11.5 and 4, 6-C<u>H</u>H), 3.98 and 4.29 (2H, m, 5-H, 7-H), 4.18 (1H, dd, *J* 11.5 and 4.5, 6-CH<u>H</u>), 5.94 (1H, ap dt, *J* 11.5 and 1.5, 2-H) and 6.42 (1H, ap dt, *J* 11.5 and 7.5, 3-H); δ_C 10.37 (C-9), 27.97 (C-8), 34.89 (C-4), 47.12 (C-6), 51.35 ($CO_2\underline{C}H_3$), 62.67 (CH_2OH), 71.31 and 75.38 (C-5, C-7), 121.30(C-2), 146.30 (C-3) and 167.24 (C-1); *m/z* (CI) 233 ($[MH]^+$, 3%), 215 (5), 201 (12), 197 (12), 183 (27), 165 (56), 147 (54), 85 (72) and 83 (100).

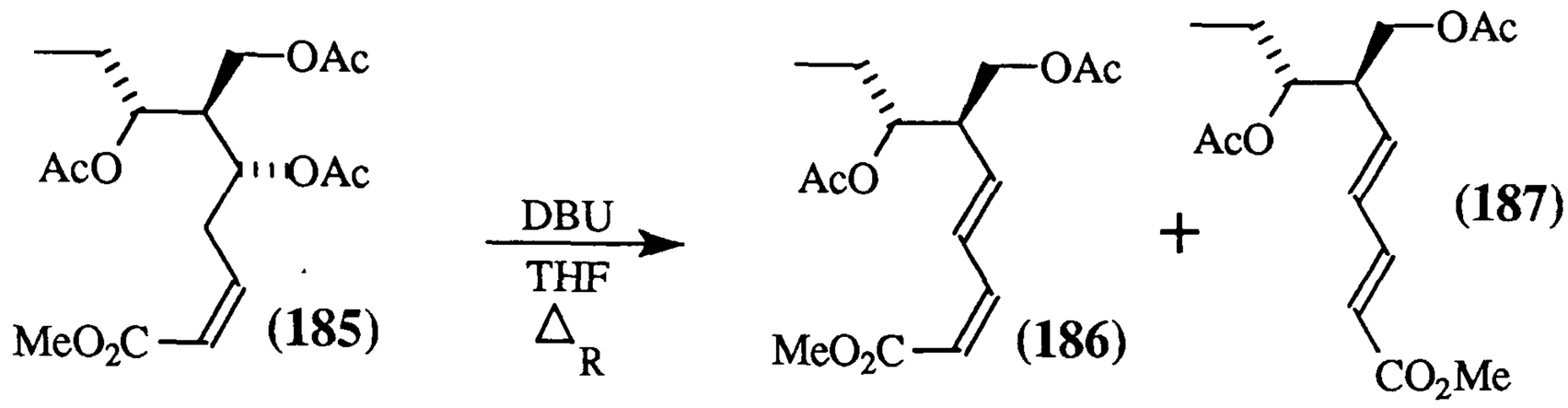
(±)-Methyl (2*Z*,5*R,6*R**,7*R**)-5,7-diacetoxy-6-acetoxymethylnon-2-enoate (185)**



Z-triol **(184)** (12mg, 0.0517mmol) was stirred in acetic anhydride (3ml) at room temperature and tosic acid (17mg) for 17h. The reaction was extracted as normal to yield a liquid (20mg). Purification by column chromatography, eluting with ethyl acetate : petrol (20:80), gave the *Z*-triacetate **(185)** as a clear liquid (27mg, >100% possible yield); R_f = 0.68 (ethyl acetate : petrol, 50:50); (M^+ absent. Found $[MH\text{-}AcOH]^+$, 299.1508. $C_{15}H_{23}O_6$ requires MH-60, 299.1495); υ_{max}/cm^{-1} 1729 and 1742 (4 x C=O); δ_H 0.89 (3H, t, *J* 7.5, 9-H_3), 1.67 (2H, m, 8-H_2), 2.02, 2.04 and 2.07 (each 3H, each s, each O_2CCH_3), 2.12 (1H, m, 6-H), 3.07 (2H, m, 4-H_2), 3.72 (3H, s, CO_2CH_3), 4.23 (2H, dd, *J* 12 and 5, 6-CH_2), 4.95 (1H, dt, *J* 7.5 and 5.5, 7-H), 5.32 (1H, td, *J* 7.5 and 3.5, 5-H), 5.89 (1H, ap dt, *J* 11.5 and 1.5, 2-H) and 6.19 (1H, ap dt, *J* 11.5 and 7.5, 3-H); δ_C 9.73 (C-9), 14.13 (C-8), 20.95 (C-4), 21.02, 22.69, 23.48 (3 x $O_2C\underline{C}H_3$), 24.67 (C-6), 43.49, 51.21, 60.86 and 61.71 (C-5, C-7, $CO_2\underline{C}H_3$, 6-CH_2), 121.96 (C-2), 148.99 (C-3), 166.39 (C-1) and 170.27, 170.51

and 170.89 (3 x $O_2\underline{C}CH_3$); *m/z* (CI) (MH^+ absent), 355 (13%), 353 (34), 339 (3), 299 ($[MH-60]^+$, 22), 239 (10), 195 (26), 125 (24), 123 (25), 111 (18), 95 (87) and 94 (100).

Reaction of the triacetate (185) with DBU



The triacetate **(185)** ('27'mg ≡ 18.5mg, 0.0517mmol) was stirred at room temperature for 0.75h in THF (3ml) and DBU (0.05ml, 0.334mmol), but no change in R_f was noted; thus the reaction was heated to reflux for 1.25h and TLC analysis indicated no starting material remained. The reaction was cooled and dilute sulfuric acid added until *p*H 3 was attained. The usual extraction procedure was followed, albeit acidic, to give a bright brick red semi-solid (50mg). Column chromatography, using ethyl acetate : petrol (10:90), returned a pale yellow liquid containing products tentatively identified as the diacetates **(186)** and **(187)** (4.8mg, 0.0161mmol, 31%); *m/z* (CI) 299 ($[MH]^+$, 9%), 257 (7), 239 (5), 238 (18), 194 (58), 179 (4), 146 (32), 119 (28), 96 (94) and 94 (100). ^1H and ^{13}C NMR spectra and MS did not enable complete identification (the high resolution MS of the starting material **(185)** did not give the molecular ion, but eliminated to a diacetate, $[MH-AcOH]^+$ = 299.1508, whilst the mixture of **(186)** and **(187)** did not give a molecular ion from high resolution MS); the ^1H NMR spectrum is included in the Results and Discussion (section 2.4).

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STUDIES TOWARDS THE SYNTHESIS OF 1β,2α-DIMETHYL GIBBERELLINS

CHAPTER FOUR:

Introduction, Results, Discussion and Conclusions

4 Introduction, Results, Discussion and Conclusions

4.1 General Introduction

Year	1850	1900	1950	1960	1992	2000
Population (x 10^9)	1.1	1.5	2.5	3.1	5.3	(6)

Table 4.1: Global population figures and prediction for the millenium[1].

The worlds' population has grown following a exponential curve, whilst food production has only increased in a linear manner, as predicted by Malthus last century. Various factors such as protectionism and war as well as poor methods of cultivation, storage or distribution have hindered efforts to eradicate hunger, such that 26 countries depended on external food aid in 1992[2].

Approaches to arable food production improvements post World War Two have advocated high intensity farming, with concurrent high usage of organochemicals, which frequently have a negative environmental impact. For example, pesticides, herbicides and fertilizers may be toxic to both flora and fauna, require financial outlay, need equipment for dispersion and long term effects on food chains are unknown[3]. Used at a high level, as in much of the developed world, organonitrates and phosphates can cause stem weakness and lower the quality of grains, and with resistance to pesticides increasing, the chances that novel chemicals will enable further production enhancement appear slim[3,4]. Thus the use of genetically produced hybrids has been developed which are high yielding, e.g., rice breed IR8, but these tend to require much use of chemical treatment and also have very low resistance to fungal or microbial overgrowth[5]. Also, the breeding programmes are long term and tend to be based in developed countries.

Thus the use of biological control agents has been investigated, from the use of predator animals such as spiders to control insects[6], to the use of plant growth regulators[7], such as natural plant hormones, hormone mimics, hormone antagonists, growth retardants, growth inhibitors, defoliants, desiccants, ripening agents or hormone

transport inhibitors. The natural phytohormones are: auxin, which is indole acetic acid (first identified as an active agent in oat coleoptiles by Charles and Frances Darwin in 1867); the gibberellins; cytokinins, which are purines derived from nucleic acids, e.g., zeatin (identified in 1968); abscisic acid, a terpene-derived unsaturated acid (isolated in 1963); and ethylene gas, (first isolated as an emittant from apples in 1934)[8]. All have a variety of effects at low concentration, and often work in synergy; however both auxins and gibberellins enhance stem elongation by increasing the internodal distance (and cytokinins by improving cell division rates) and this is the principal area of application for enhanced food production, so that crops may grow faster towards light than weeds. Numerous synthetic analogues have been made of the various phytohormones, but especially the auxins, such as Agent Orange.

4.2 Discovery and Structure of Gibberellins

The bakanae ("foolish seedlings") disease of rice, when excessively tall plants were obtained that gave poor crop yields, was first noted in Japan. Initially scientists in the 1920's at Tokyo University isolated the fungal culture which caused the disease as *Fusarium moniliforme* (the imperfect form of *Gibberella fujikuroi*); the active compound gibberellic acid (GA_3) was first crystallised in 1935 and identified as a tetracyclic diterpenoid secondary metabolite, with two more gibberellins characterised in 1938[9,10,11]. In 1954 (in Britain) a gibberellin was isolated directly from a higher plant, and identified as being the same as the third Japanese product isolated and named GA_1[8]; the structure was determined in 1958[12].

To date 108 gibberellins[13], numbered sequentially upon discovery, have been found in a variety of fungi and eucaryotic plants, although only a few are available commercially ($GA_{3,\ 4,\ 7,\ 13}$), but the first gibberellin identified in plants (GA_1) (**1**) is not commercially available[11]. Total syntheses of gibberellins have been reported, but as with the steroids these are lengthly and of very low overall yield[14], hence the commercially available compounds are used in semi-synthetic procedures to obtain target molecules[15]. The numbering system (the C_{20} gibberellin is numbered as an example) is based upon the gibberellane skeleton which is itself based upon steroid

structure numbering, but all the natural gibberellins have the enantiomeric stereochemistry to gibberellane, so are prefixed *ent*- to invert all the positions relative to gibberellane[16].

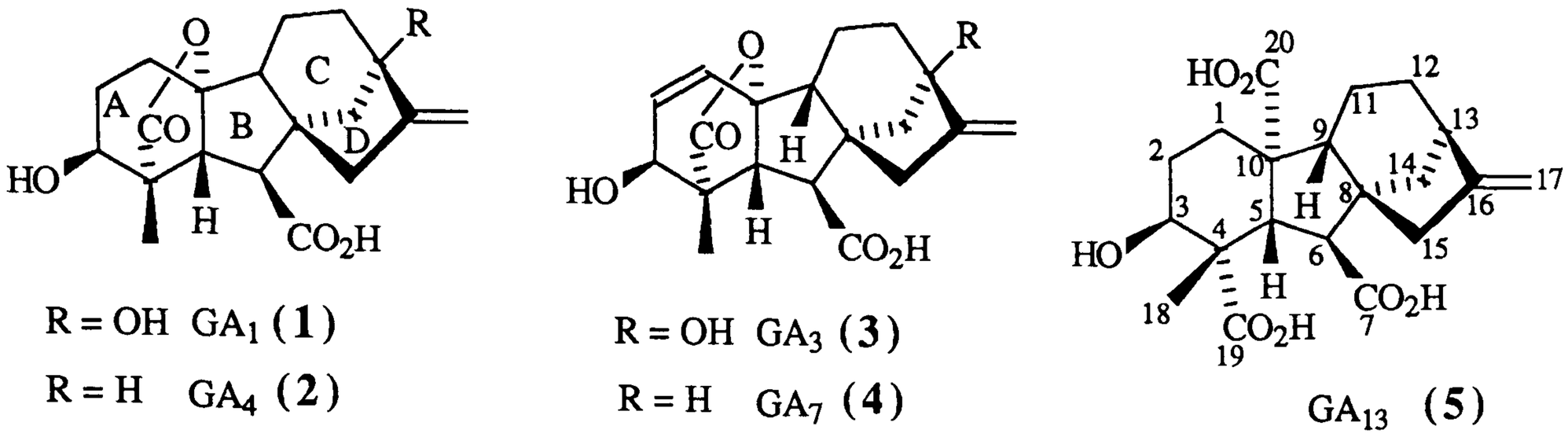
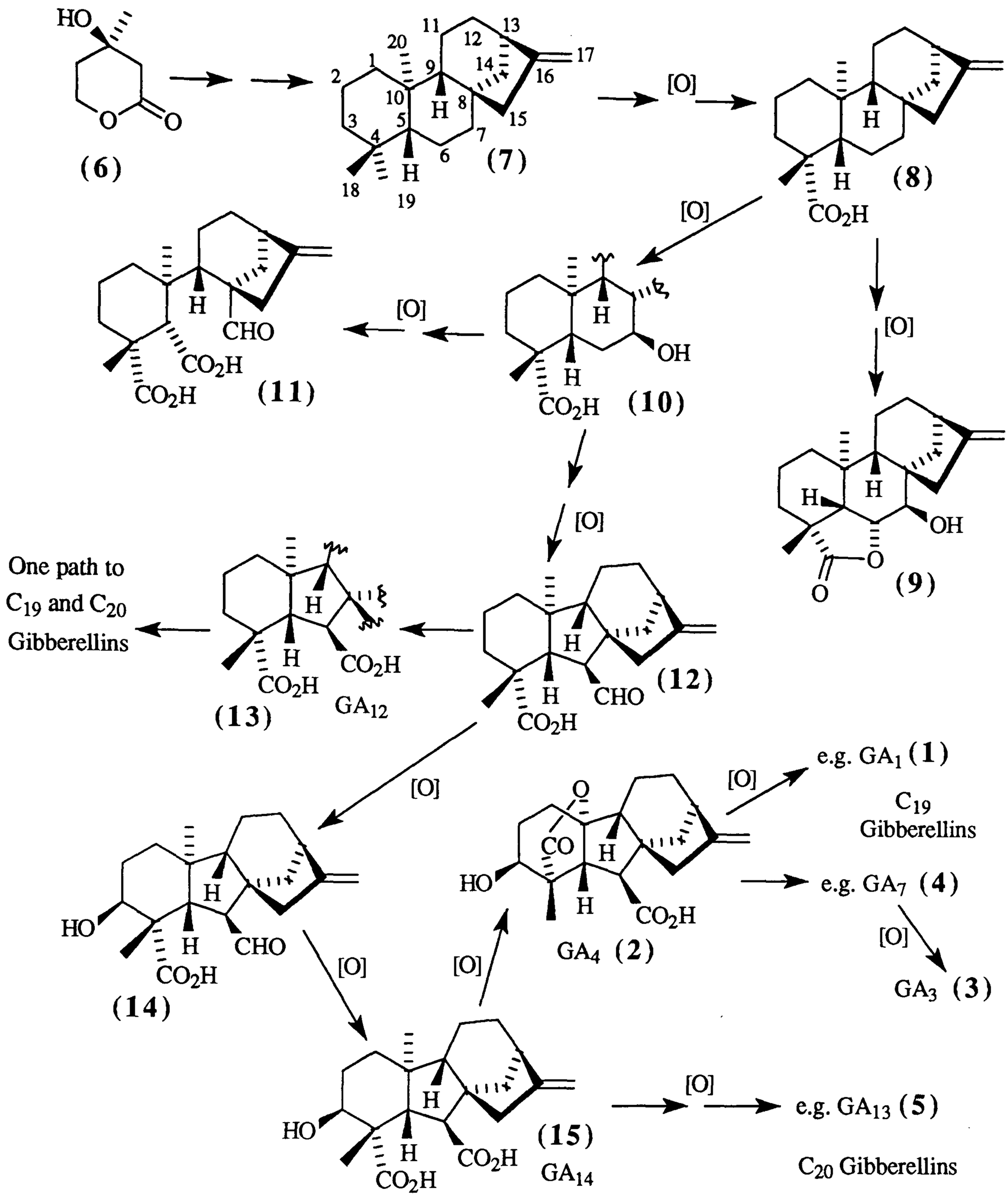


Figure 4.1: Structure of common C_{19} and C_{20} gibberellins.

4.3 Biosynthesis of Gibberellins

The biosynthesis of gibberellins has been extensively reviewed[17-20]. The first tetracyclic precursor is *ent*-kaurene (**7**) which is derived from mevalonic lactone (**6**), and formation of (**7**) follows the same path in all eucaryotes (scheme 4.1)[21]. Variations in the biosynthetic pathways and metabolites between different organisms follow from (**7**), but all are based largely upon hydroxylation and oxidation; the path denoted is that from *G. fujikuroi* [22].

The C-19 of (**7**) is sequentially oxidised to *ent*-kaurenoic acid (**8**) where it may be cyclised via a 6,7-epoxide to the kaurenolides, e.g., (**9**) or oxidised at the 7 position to a *beta* alcohol (**10**). The latter may be oxidised to the seco-ring B acids (**11**) or undergo a ring contraction to GA_{12} 7-aldehyde (**12**); the aldehyde is either then oxidised to the acid (GA_{12}) (**13**) which leads to further C_{20} and C_{19} gibberellins, or hydroxylation occurs at C-3 to give (**14**) (which is typical of fungi, in higher plants 13-hydroxylation tends to occur at the equivalent point in the synthesis[11]). The aldehyde functionality of (**14**) is then oxidised to form C_{20} gibberellin dicarboxylic acids, e.g., GA_{14} (**15**), for further gibberellin anabolism. Sequential oxidation of the 20-methyl *via* an alcohol gives an aldehyde which is the immediate precursor to the more prolific and potent C_{19} gibberellins, e.g., (**2**). From the many side reactions, the possibilities of isolating many more kaurenes or gibberellic acids are numerous, as new species of plants are investigated.



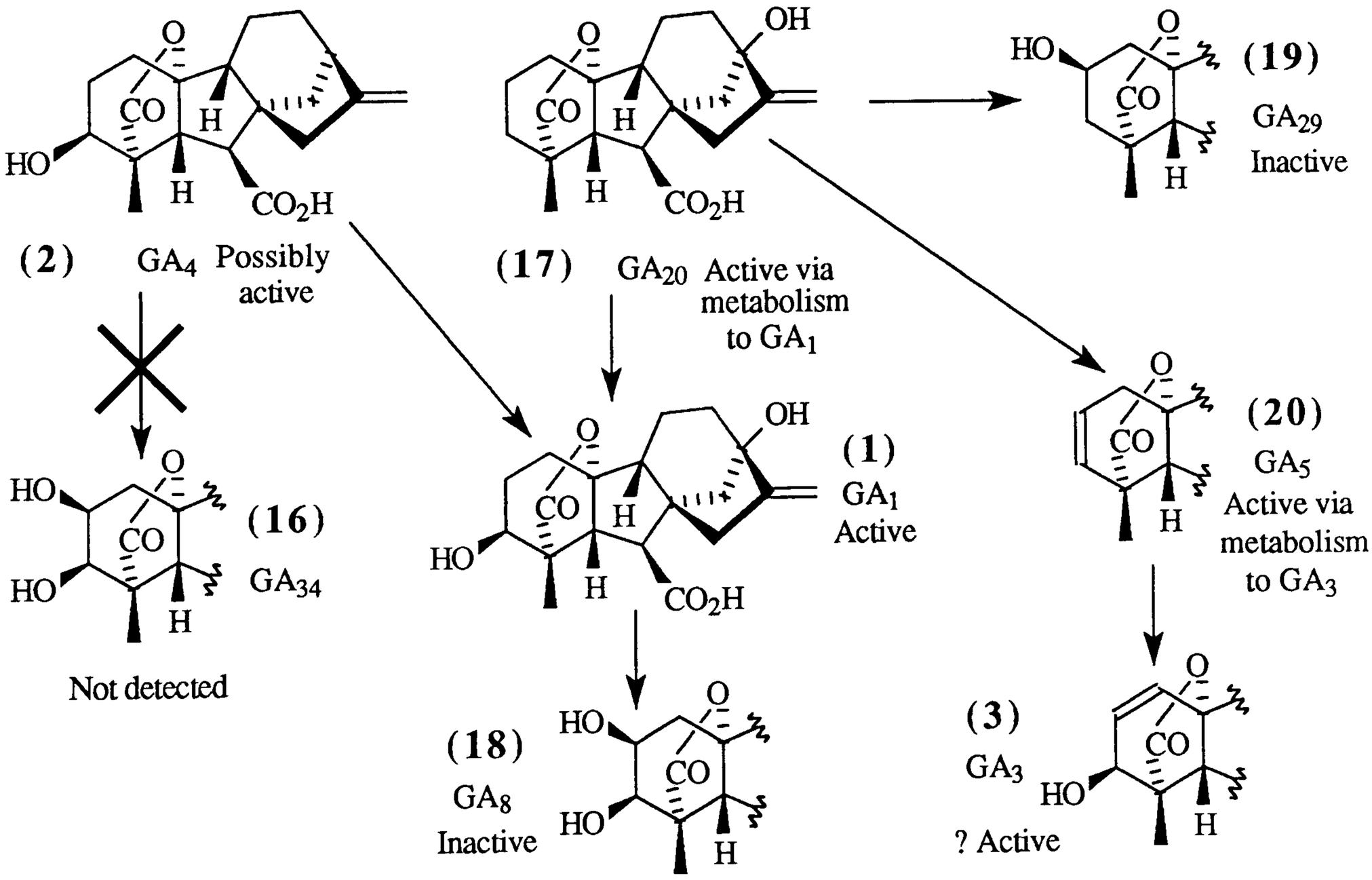
Scheme 4.1: Overview of biosynthesis of gibberellins in *G. fujikuroi*.

4.4 Hydroxylation and Catabolic Studies

Gibberellins are involved in the control of much plant development and growth[14,17-19]. However, some features added at various positions of the *ent*-gibberellane skeleton confer much improved biological activity[20-22]: a 3β-hydroxyl derivative is more active than if an α-OH or no hydroxyl is present, and a free 7-carboxylic acid is necessary. A 19,10 γ-lactone imparts high activity, the precursor C_{20}

gibberellins have lower activity than their C_{19} successors. The presence of the 13-hydroxyl has not been proved as a necessity for activity.

GA_1 **(1)** has been suggested to be the only active GA in higher plants from studies using dwarf mutants of pea and maize[9,10], but GA_3 **(3)**, which shows similar activity in bioassays has been found in the stem fluids of *Carica papaya*[23] (papaya) and *Camellia sinensis* (tea)[24]. Whilst GA_4 has been found in higher plants *in vivo*[25], it is metabolized in *Zea mays* (maize, aka corn) to GA_1[26] and the (inactive) 2β-hydroxyl metabolite GA_{34} **(16)**, has not been found, so GA_4 is not proven to be inherently bioactive. Although hydroxylation can occur *in vivo* at C-13 and on any primary or secondary carbon except at C-14[27], the pattern of hydroxylation depends on the particular tissue or species concerned[28]; however, at the end of the useful biological role of the GA, the catabolic pathway is entered where the gibberellins are generally 2β-hydroxylated, an irreversible method of complete deactivation prior to further degradation[29]. As the mode of deactivation of GA_3 is unknown, this is part of the circumstantial evidence to support the theory that only GA_1 is biologically active.



Scheme 4.2: Metabolism of C_{19} gibberellins in *Z. mays*.

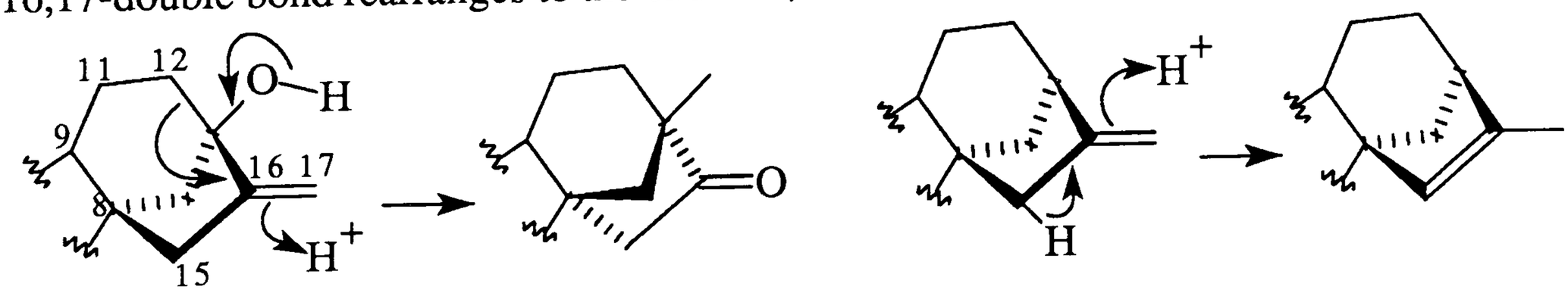
Hydroxylation at C-1 occurs also, e.g., GA_{60} and GA_{61} (which have 1β-OH) are found in *Triticum aestivum* (wheat)[25], and GA_{16} (which is 1α-hydroxy GA_4) found in *G. fujikuroi*, but as these have lower activity than their non-hydroxylated precursors, whether these are degradation products, unwanted metabolites or useful hormones has not been determined[30]. Other positions of hydroxylation and their effects on flora have been surveyed by Graebe but whilst these show little difference to the non-hydroxylated parent structures on stem elongation[31], they have effects on other functions in higher plants (e.g. flowering)[26,27].

4.5 Applications and Commercial Uses

As noted, the gibberellins cause stem elongation and can cause inhibition of flowering in dicotyledons[9,26]; but other effects include breaking winter dormancy of seeds by inducing the synthesis of α-amylase, (which is used in the brewing industry upon barley to make the wort), promoting fruit growth in grape plants, preventing pre-harvest drop of apples, enhancing the actions of other phytohormones (or used as alternatives to IAA for stem growth as gibberellins do not have much effect on rooting of plants)[4,8,11,22]. As global sales of agrochemicals were US $20,000,000,000 in 1989, of which plant growth regulators were only approximately 4%, the market for the commercially underapplied hormone based market can surely expand[3].

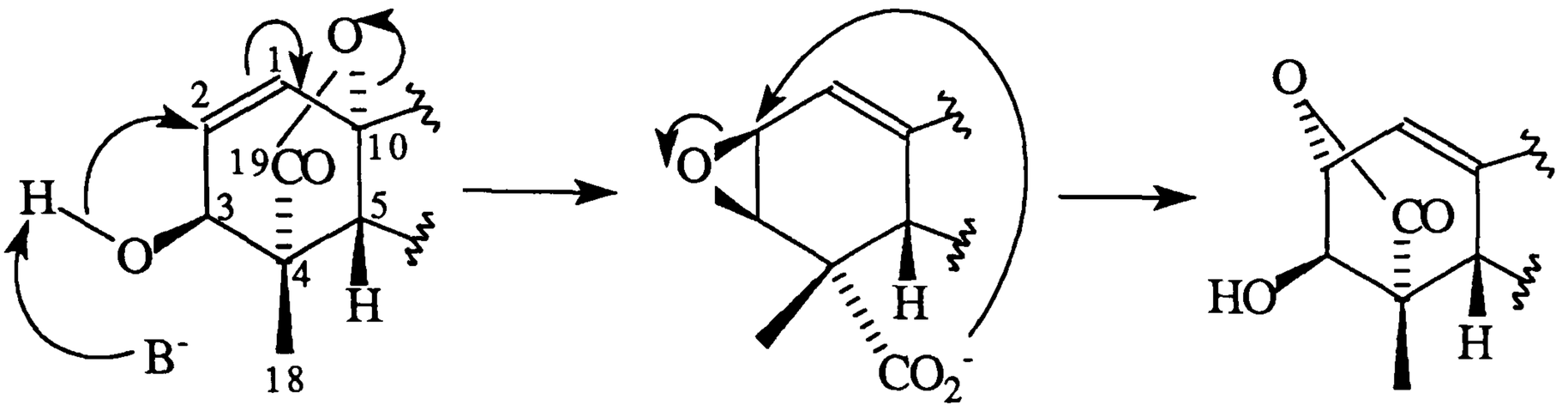
4.6 Acid and Alkali Induced Rearrangements of the Carbon Skeleton

13-Hydroxygibberellins can react with strong acids to form the keto-inverted C and D rings via a modified Wagner-Meerwein rearrangement[32]. Thus the use of dilute hydrochloric acid in work-up procedures is required. In 13-dehydro gibberellins, the 16,17-double bond rearranges to the endo-15,16-olefin.



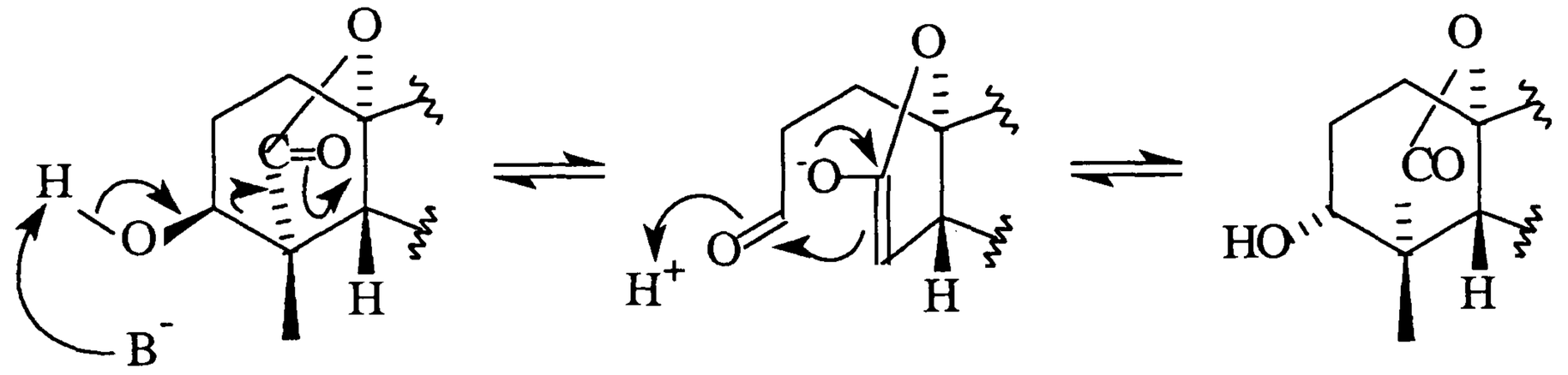
Scheme 4.3: Acid catalysed rearrangements of C and D rings of 13-OH gibberellins.

The presence of a 3β-OH in an allylic form, as in GA_3, may facilitate reaction with base to eliminate the carboxylate via the epoxide, which undergoes nucleophilic attack by the carboxylate anion to give the the 19,2-lactone[33,34].



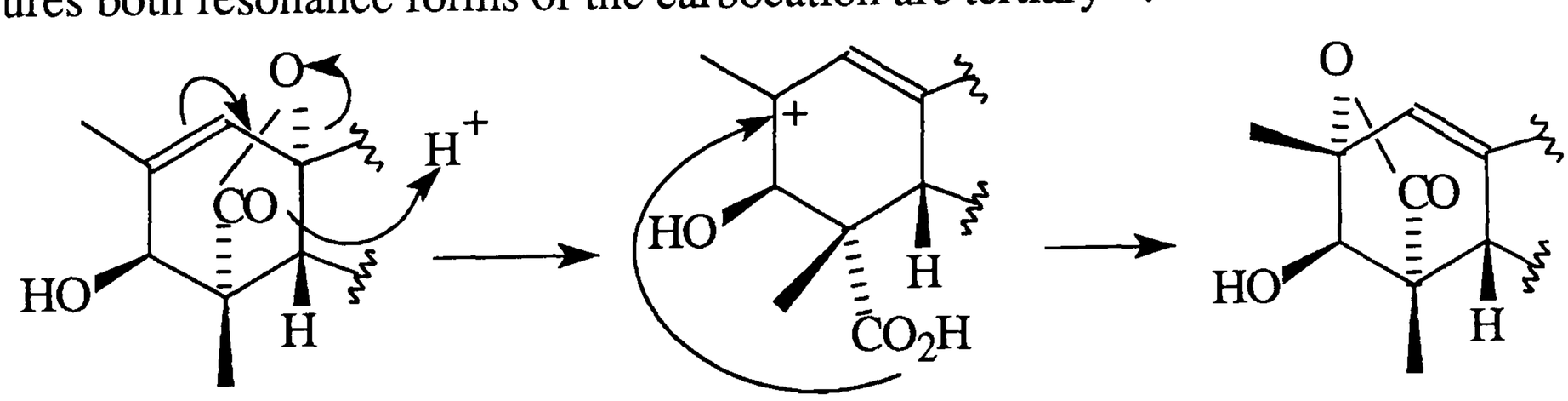
Scheme 4.4: Base catalysed rearrangements of ring A of an allylic lactone.

A non-allylic 3β-OH on ring A of 19,10γ-lactones can also rearrange with base, but to the thermodynamically favoured 3α-epimers; the mechanism is a retro-aldol / aldol rearrangement[35].



Scheme 4.5: Base catalysed rearrangements of 3β-hydroxy C_{19}-gibberellins.

Acid derived rearrangements of the A ring are possible also, the allylic stabilisation of the carbocation formed enables formation of the isolactone. However, the reaction occurs far more easily with alkyl substitution at the 2 position, as this ensures both resonance forms of the carbocation are tertiary[36].



Scheme 4.6: Acid-catalysed rearrangement of ring A.

4.7 The Effects of 1- and 2- Substituents upon Biological Activity.

3β-Hydroxylated C_{19} gibberellins containing a 2α-methyl, 2α-ethyl or 1β-methyl group have a ten to hundred fold increase in potency compared to the non-alkylated parent, and have been labelled as "superactive"[29,36,37,38]. However, the equatorial 2β- or 1α- alkyl diastereomers have similar bioactivity to the natural precursor[38,39] (figure 4.2). Larger alkyl groups at C-2 produce GA derivatives with little biological activity, possibly due to excessive lipophilicity, but no correlation could be found between size of alkyl group and activity[36].

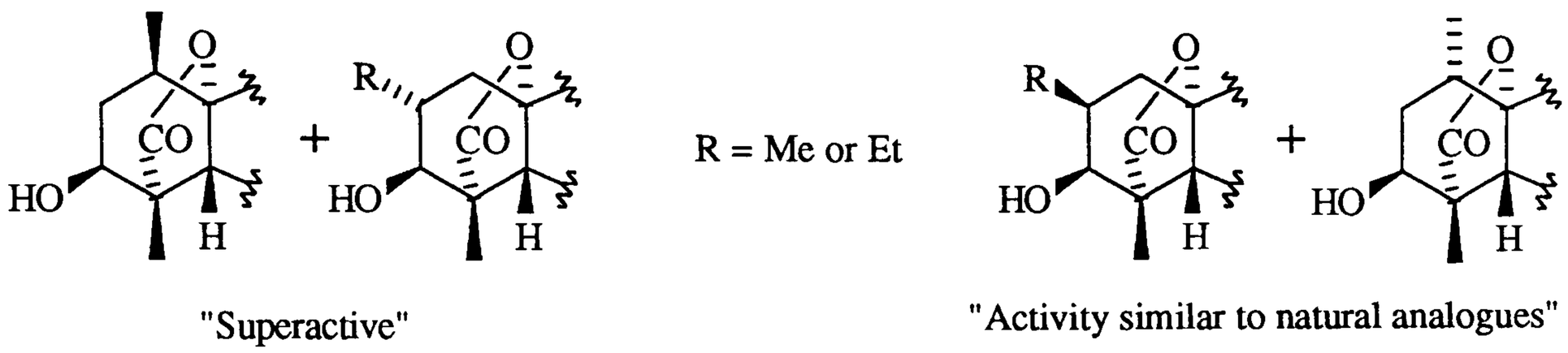


Figure 4.2: Effect of ring A substituents on bioactivity of GA_1 derivatives.

Only three 2,2-dialkylated gibberellins have been synthesized: the 2,2-dimethyl derivatives of GA_1[40] **(21)** and GA_4[37,40] **(22)** and 2,2-diethyl GA_4[37,40] **(23)** (figure 4.3). The dimethyl derivatives are more active than the non-substituted precursors but not as potent as the mono axial substituted 2α-methyl compounds **(24)** and **(25)**[29,37,39] (figure 4.4); however the diethyl product **(23)** caused negligible improvement in growth compared to GA_4 **(2)** in either maize or lettuce cotyledons[39].

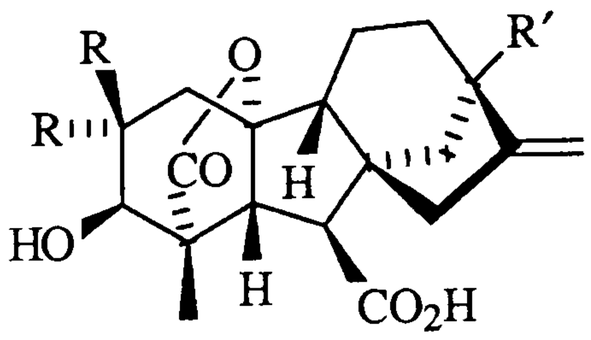
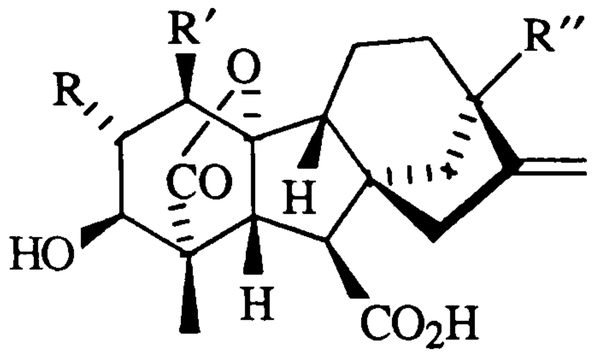


Figure 4.3: Previously prepared 2,2-dialkylated gibberellins.

The difference between the "superactive gibberellins" and those of a non-enhanced status may be due to the rate of metabolism to inactive products, as bioassays with *Z. mays* have shown the molecules to have more prolonged rather than more rapid activity[41]. Oxidation of gibberellins to the 2β-hydroxylated derivatives (e.g. GA_1 to

GA_8, scheme 4.2) has been shown to occur with retention of configuration[42], so it would seem that the action of alkyl substituents is not to block directly the oxygenation, but to either prevent the enzyme proteins binding closely enough, or more probably to cause a conformational change in the A ring: axial substituents at C-1 or C-2 cause flattening of the ring compared to the non-substituted ring in GA_1[43], which may cause the molecule not to fit into the active site.



R = Me R′ = H R″ = H **(24)**

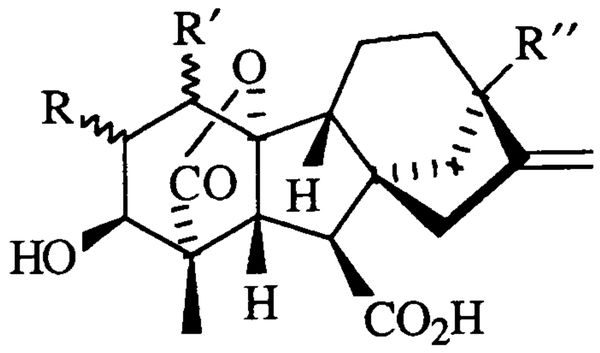
R = Me R′ = H R″ = OH **(25)**

R = H R′ = Me R″ = H **(26)**

R = H R′ = Me R″ = OH **(27)**

Figure 4.4: Gibberellin analogues with either a 1β or 2α-methyl group.

However, a gibberellin analogue with both 1β-methyl and 2α-methyl (ie both diaxial) substituents has not been prepared. 1β,2α-Dimethyl GA_1 (**28**) was the initial target of this project as a potential "superactive" gibberellin; however, whether the effect of two axial groups would flatten ring A and thus cancel each others effects, cause an additive effect or give the desired synergy can only be determined upon bioassay; the GA_4 (**29**) analogue was a second target. The diequatorial substituted compounds, which would be interesting developments of the mono-equatorial derivatives were also required (figure 4.5).



R = α-Me R′ = β-Me R″ = OH **(28)**

R = α-Me R′ = β-Me R″ = H **(29)**

R = β-Me R′ = α-Me R″ = H or OH Diequatorial isomers

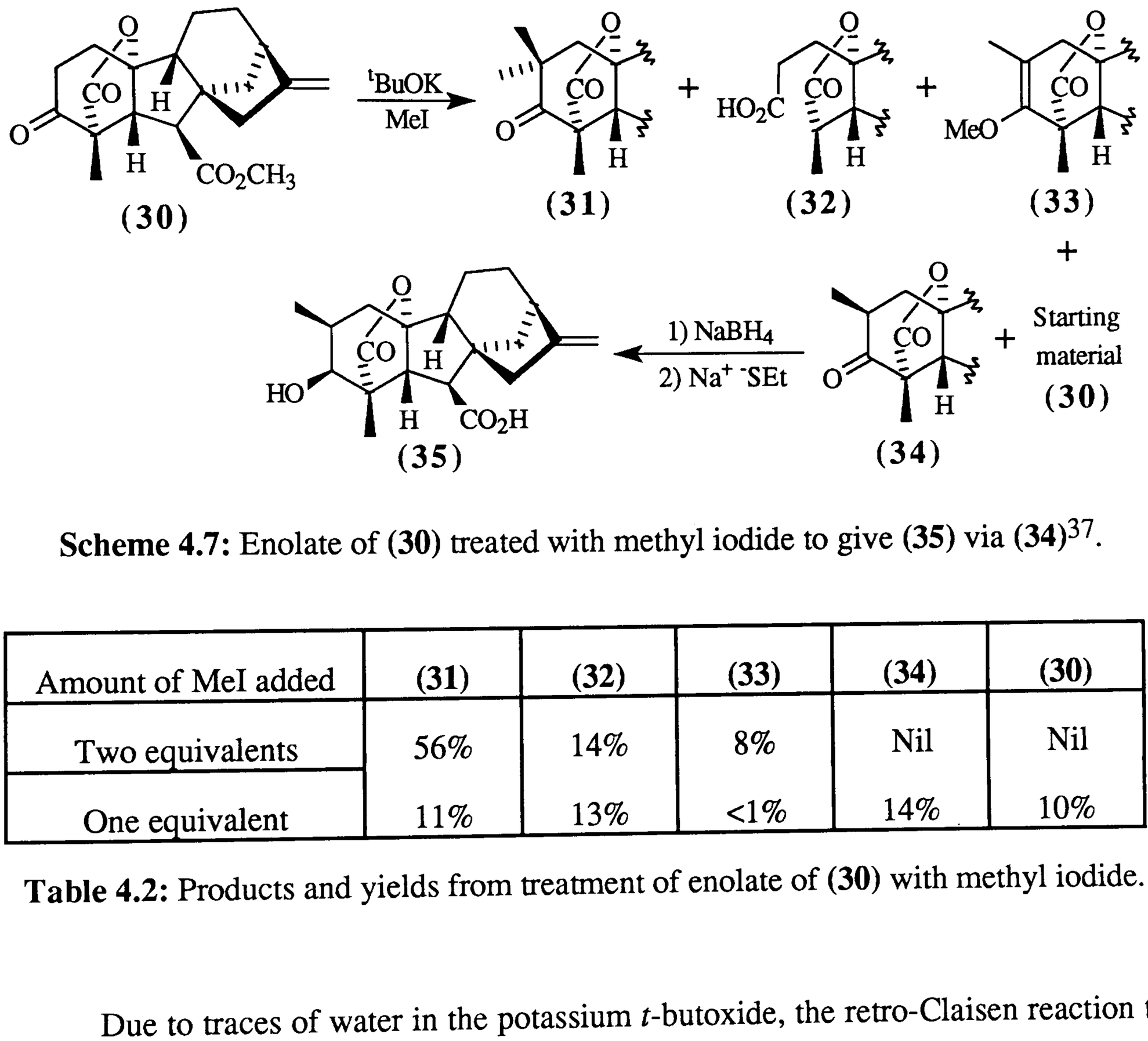
Figure 4.5: Target 1,2-dimethyl gibberellins.

4.8 Previous Syntheses of 1- and 2- Alkylated Gibberellins

Use of 3-oxo-GA_9 methyl ester (**30**) enabled formation of an enolate, which could be quenched with a variety of electrophiles to form 2-alkylated derivatives. Reaction of (**30**) with two equivalents of potassium *t*-butoxide and methyl iodide gave

the 2,2-dimethyl-3-oxo-GA_9 methyl ester (**31**), plus the A-ring seco-acid (**32**) and the enol ether (**33**)[37] (scheme 4.7 and table 4.2). Sodium borohydride reduction of (**31**) gave the 3β-alcohol (the desired stereoisomer) which on de-esterification gave 2,2-dimethyl GA_4 (**22**).

Repeating the experiment using only one equivalent of methyl iodide gave the desired product, 2β-methyl-3-oxo-GA_9 methyl ester (**34**), accompanied by three unwanted by-products (scheme 4.7 and table 4.2). Reduction of the ketone of (**34**) with sodium borohydride followed by de-esterification gave 2β-methyl GA_4 (**35**).

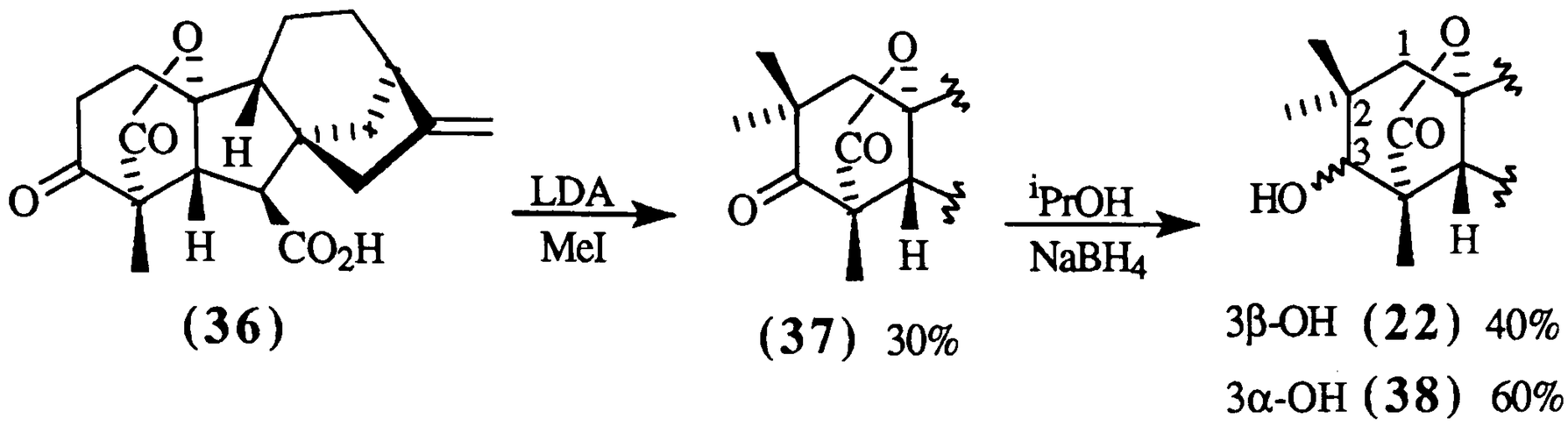


Scheme 4.7: Enolate of (**30**) treated with methyl iodide to give (**35**) via (**34**)[37].

Amount of MeI added	(31)	(32)	(33)	(34)	(30)
Two equivalents	56%	14%	8%	Nil	Nil
One equivalent	11%	13%	<1%	14%	10%

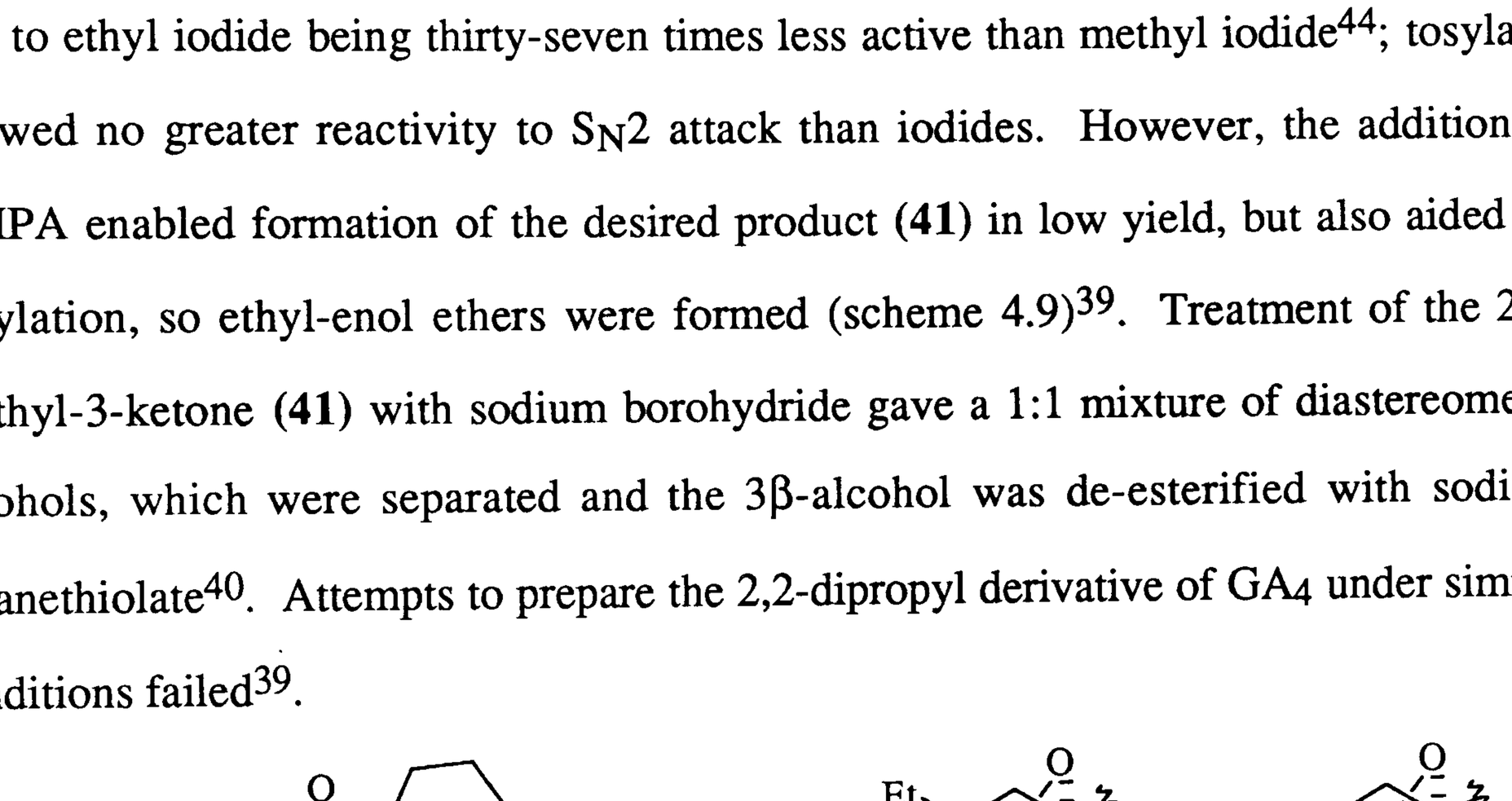
Table 4.2: Products and yields from treatment of enolate of (**30**) with methyl iodide.

Due to traces of water in the potassium *t*-butoxide, the retro-Claisen reaction to form (**33**) was possible; to avoid this, treatment of the free acid (**36**) with LDA and methyl iodide gave (**37**) as the sole product; the ketone was reduced with sodium borohydride to give a mixture of the diastereomers (**22**) and (**38**) (scheme 4.8)[40].



Scheme 4.8: Preparation of 2,2-dimethyl GA_4 **(22)**.

Due to the higher bioactivity of 2,2-dimethyl GA_4 **(22)** compared to GA_4 **(2)**, other dialkylated products were required for bioassay. Treatment of 3-oxo-GA_9 methyl ester with LDA followed by ethyl iodide returned only starting material[39]. This may be due to ethyl iodide being thirty-seven times less active than methyl iodide[44]; tosylates showed no greater reactivity to S_N2 attack than iodides. However, the addition of HMPA enabled formation of the desired product **(41)** in low yield, but also aided *O*-alkylation, so ethyl-enol ethers were formed (scheme 4.9)[39]. Treatment of the 2,2-diethyl-3-ketone **(41)** with sodium borohydride gave a 1:1 mixture of diastereomeric alcohols, which were separated and the 3β-alcohol was de-esterified with sodium ethanethiolate[40]. Attempts to prepare the 2,2-dipropyl derivative of GA_4 under similar conditions failed[39].

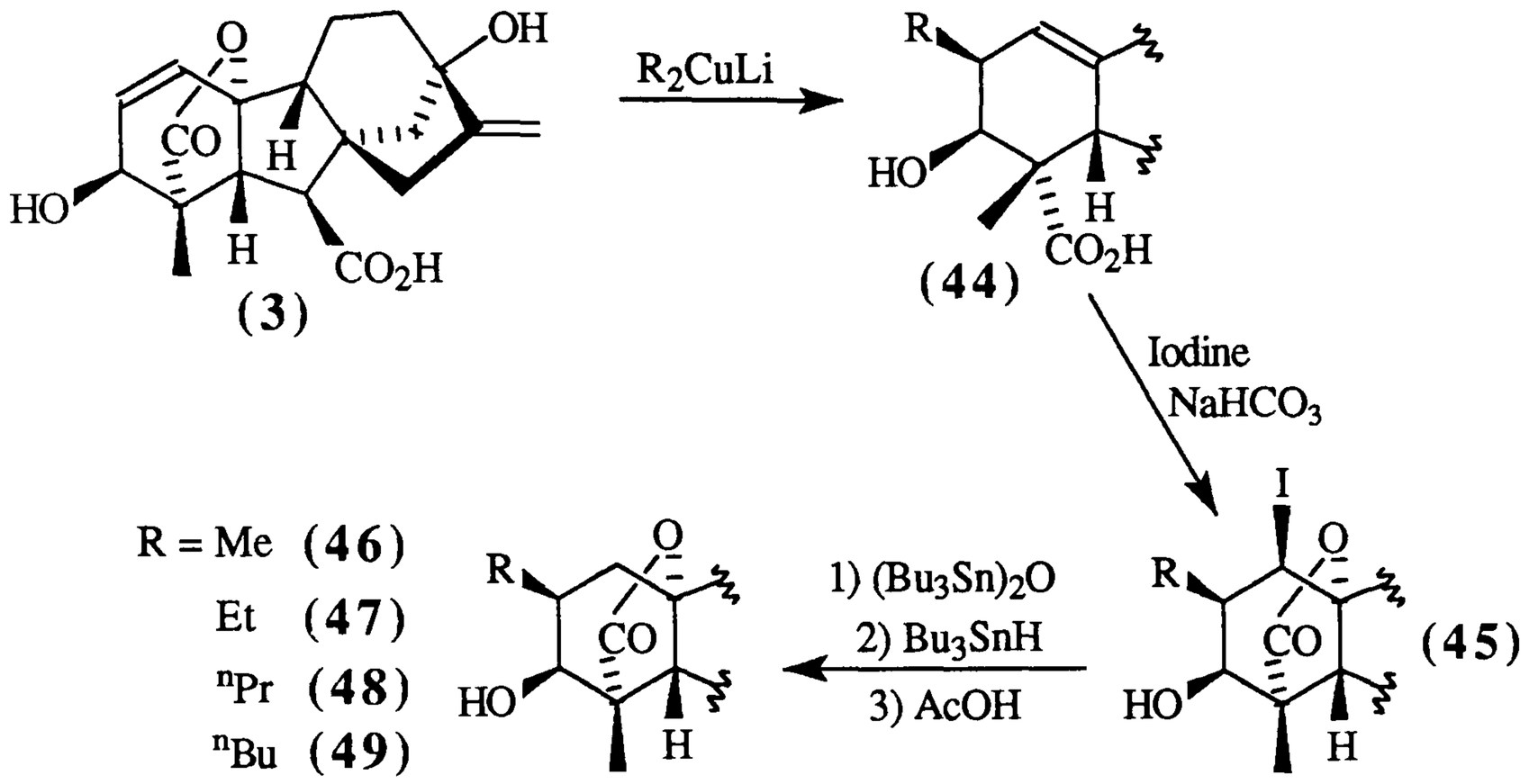


Scheme 4.9: Synthesis of 2,2-diethyl GA_4 **(23)**[39].

Treatment of 3-oxo-GA_{20} methyl ester (the 13-hydroxyl analogue of 3-oxo-GA_9 methyl ester **(30)**) with LDA and methyl iodide gave a complex mixture of products. It

is not evident why a 13-hydroxyl group should be interfering with this reaction, but even when the alcohol was protected, a complex mixture of products was formed in the enolate reaction. To overcome the problem, 2,2-dimethyl-GA_1 (**21**) was prepared by incubation of 2,2-dimethyl-GA_4 (**22**) with *G. fujikuroi* mutant B1-41a (which can hydroxylate at the 13 position) in 60% yield[40].

Due to the inherent problems with this enolate chemistry to prepare monoalkylated gibbrellins, an alternative approach was investigated.



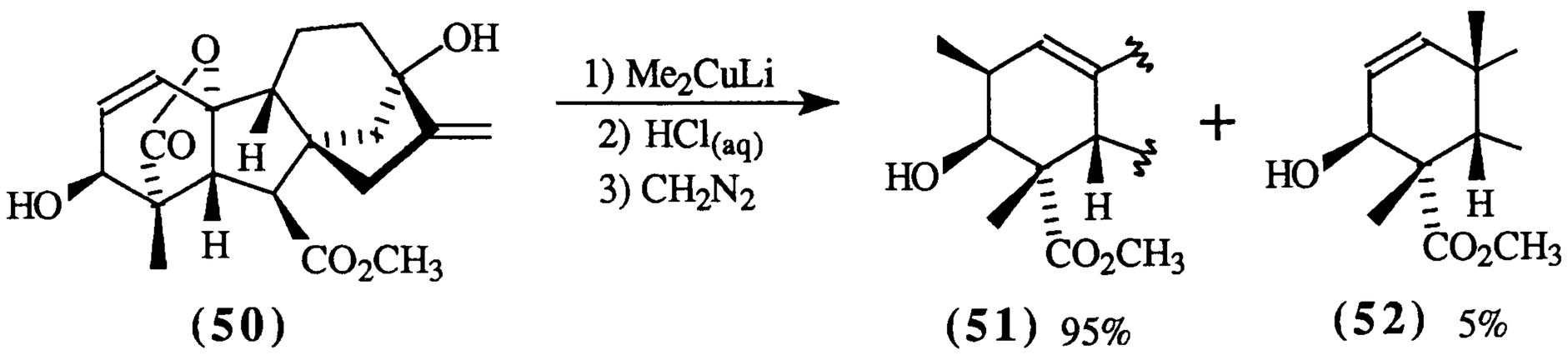
Scheme 4.10: S_N2' *anti* cuprate reactions upon GA_3 (**3**).

Reaction of (**3**) with lithium dialkyl cuprates (Gilman reagents) resulted in *anti*-S_N2' displacement of the allylic lactone to give an acid (**44**)[45]. Regeneration of the 19,10γ-lactone by an iodolactonisation procedure derived from a method by Corey[46] gave (**45**), which, via a radical reduction on the stannane ester, gave 2β-alkylated gibberellins (**46**) to (**49**). Whilst the 2β-methyl acid (**46**) formed in 100% yield, attempts to form larger 2β-alkyl derivatives were limited by steric effects, as seen in table 4.3; the increase in *syn* products may be due to interactions between the 3β-hydroxyl and the cuprate. Thus an alternative route to addition of more bulky alkyl groups was required, especially for secondary and tertiary alkyl groups, which did not add under these conditions[39].

R group	Me	Et	^{n}Pr	^{n}Bu
% *Anti* addition	100	90	70-80	60
% *Syn* addition	0	Trace	10-15	30-40

Table 4.3: Addition of lithium dialkylcuprates to GA_3 (**3**).

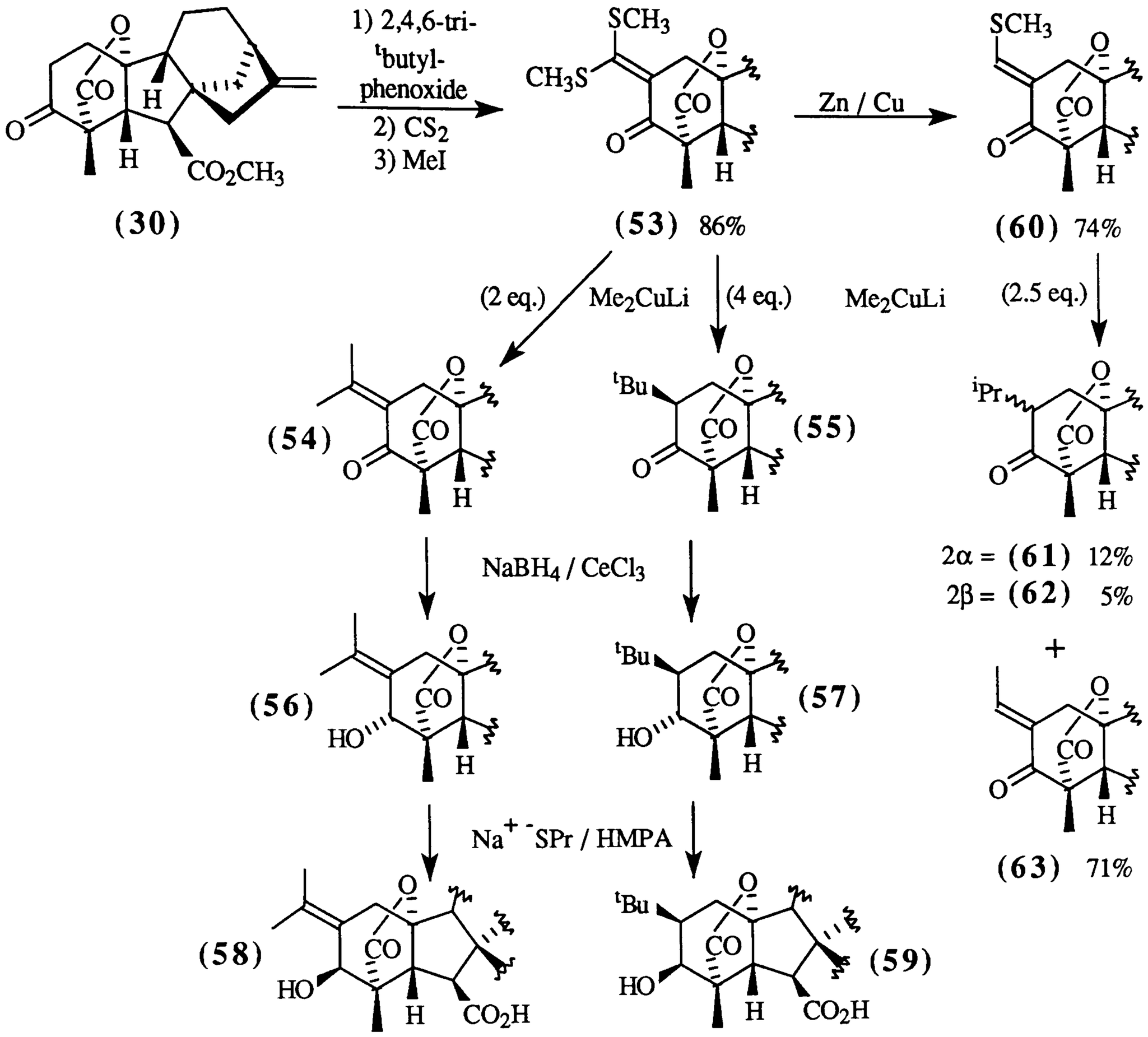
Similar work by Beale using lithium dimethylcuprate upon GA_3 methyl ester (**50**) followed by methylation *in situ* with diazomethane gave 95% (**51**) and 5% of the 10β-methyl compound (**52**), indicating some S_N2 displacement was occurring (scheme 4.11). Use of higher order cuprates increased the amount of by-products formed and conversion was no longer quantitative[47].



(**50**) (**51**) 95% (**52**) 5%

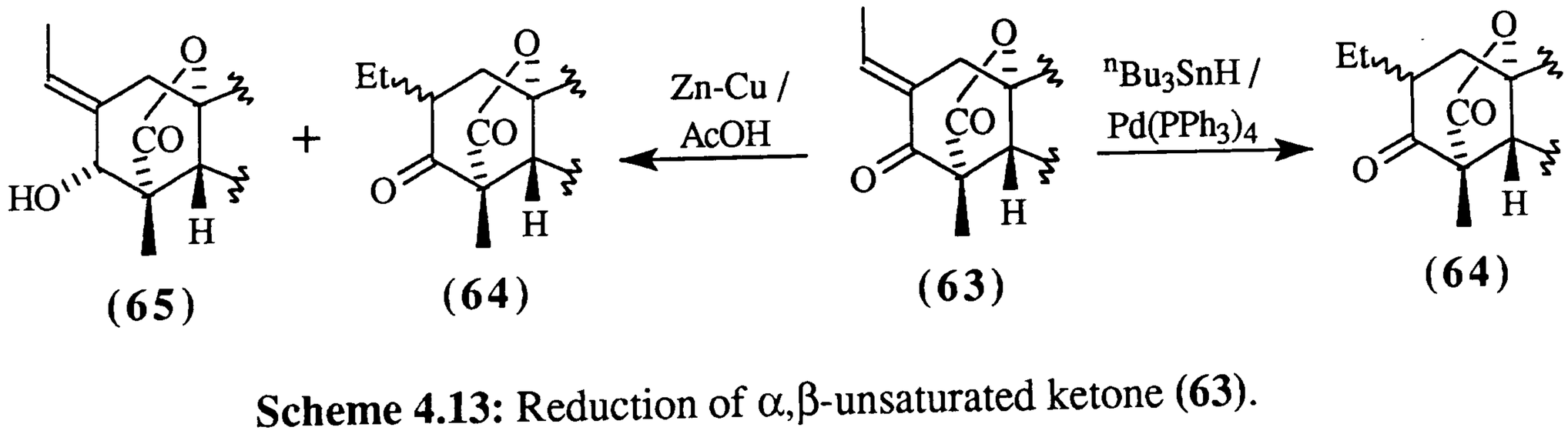
Scheme 4.11: S_N2' *anti* cuprate reactions upon GA_3 methyl ester (**50**).

Reaction of 2-bis(methylthio)methylene-GA_9 methyl ester (**53**) with two equivalents of lithium dimethylcuprate gave the dimethylated derivative (**54**), whereas with four equivalents of cuprate the 2β-*t*-butyl derivative (**55**) was the major product (scheme 4.12)[48]. Reduction of both (**54**) and (**55**) under Luche conditions gave predominantly the 3α-alcohols (**56**) and (**57**). However, on demethylation of the 7-methyl ester, epimerisation occurred at C-3 giving the required 3β-alcohols (**58**) and (**59**). Treatment of the dithiomethylene (**53**) with a zinc-copper couple gave the mono-thioenol (**60**) which on reaction with lithium dimethylcuprate, gave a mixture of both 2α-isopropyl ketone (**61**) and 2α-isopropyl ketone (**62**), and the unsaturated ketone (**63**).



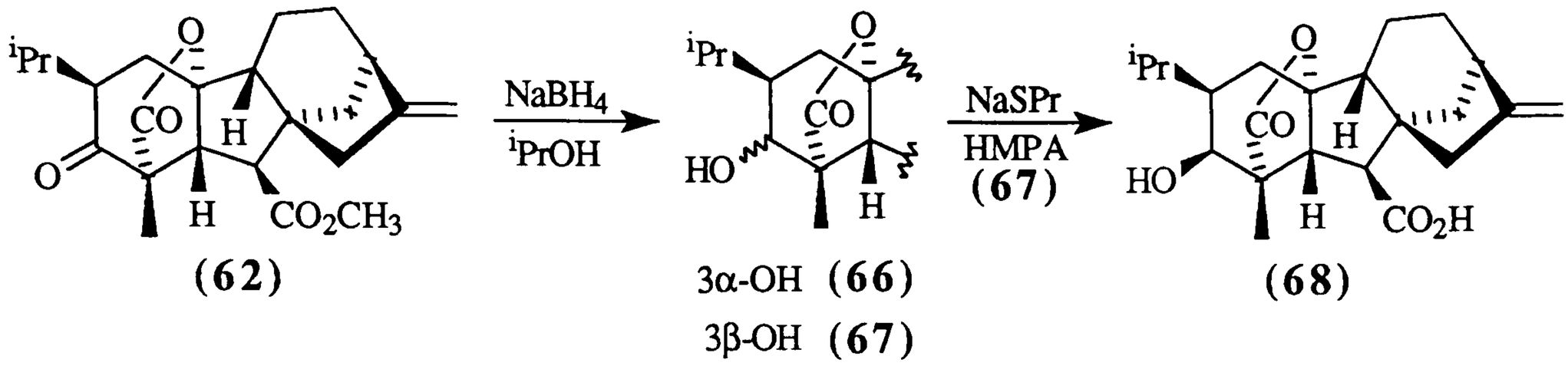
Scheme 4.12: Reactions of dithiomethylene **(53)**.

Treatment of **(63)** with $Pd(PPh_3)_4$ and $^nBu_3SnH$ gave both 2α- and 2β-ethyl-3-oxo gibberellins **(64)**. Reaction of **(63)** with a zinc-copper couple and acetic acid did not produce the expected 2α-ethyl GA_4, but yielded a mixture of 2-ethyl-3-oxo gibberellins **(64)** plus 2-(*E*)-ethylidene-3-epi-GA_4 **(65)** (scheme 4.13)[36]



Scheme 4.13: Reduction of α,β-unsaturated ketone **(63)**.

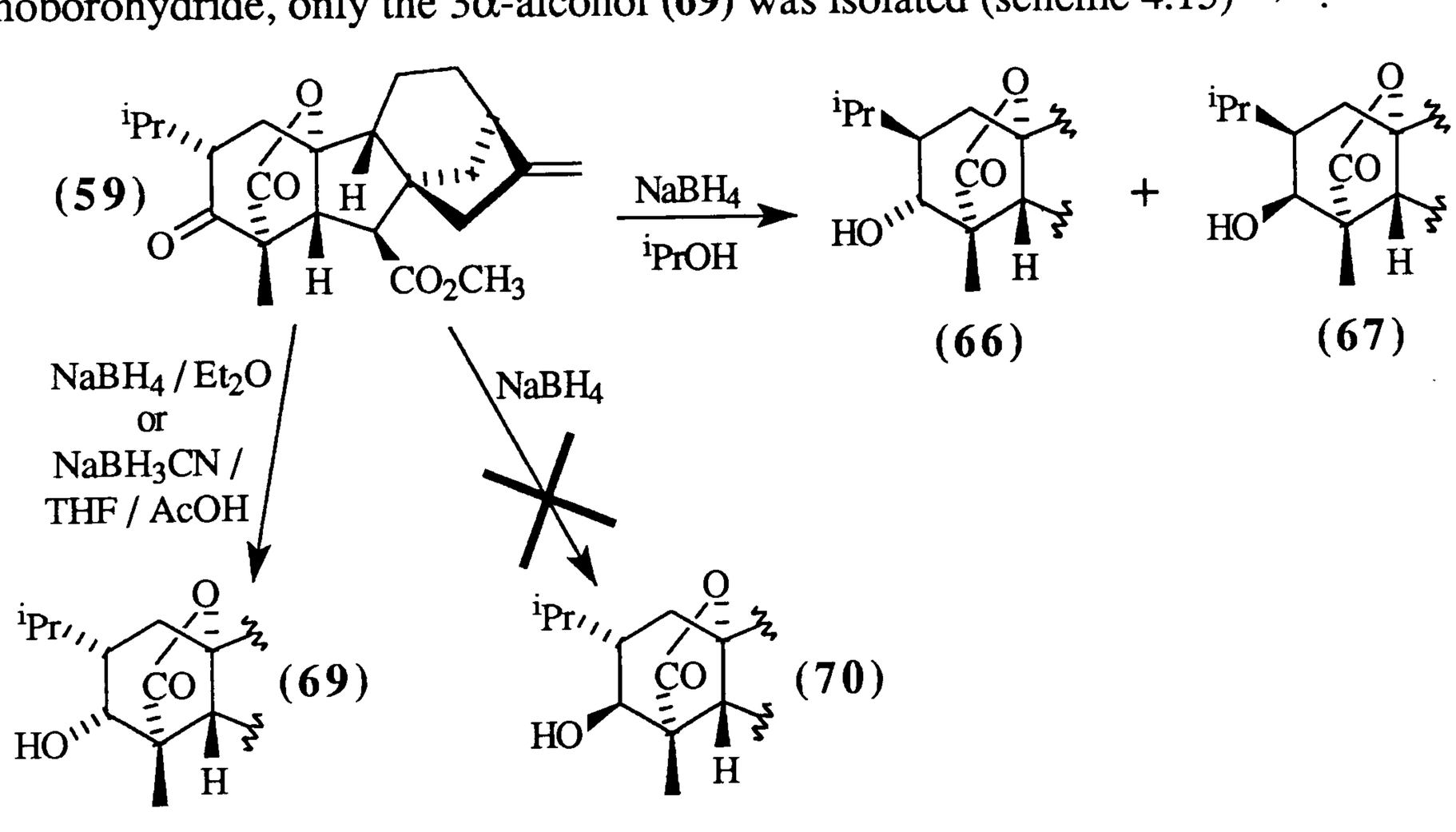
Reduction of 2β-isopropyl-3-ketone (**62**) with sodium borohydride in isopropanol gave a mixture of the 3α- and 3β- alcohols (**66**) and (**67**) which were separated by flash chromatography (scheme 4.14)[48]. Treatment of the 3β-alcohol (**67**) with sodium propanethiolate gave the required 2β-isopropyl GA_4 (**68**).



(**62**) (**68**)

Scheme 4.14: Preparation of 2β-isopropyl GA_4 (**68**)[48].

Attempts to reduce 2α-isopropyl-3-ketone (**61**) to the corresponding 3β-alcohol (**70**) were unsuccessful. Treatment of (**61**) with sodium borohydride in isopropyl alcohol led to isomerisation at C-2, giving the 2β-isopropyl-3-alcohols (**66**) and (**67**). When the reduction was repeated using sodium borohydride in ether or with sodium cyanoborohydride, only the 3α-alcohol (**69**) was isolated (scheme 4.15)[36,48].

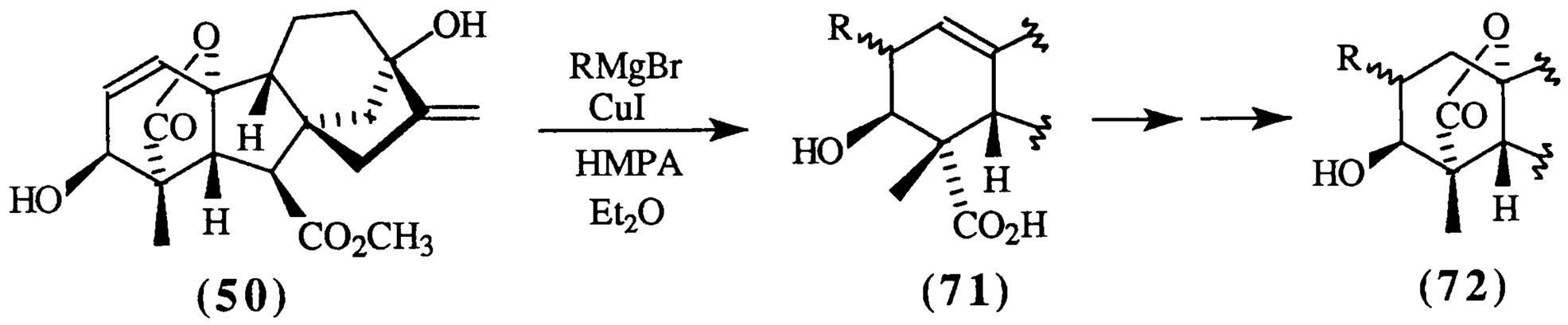


Scheme 4.15: Attempted reductions of 2α-isopropyl ketone.

Using the methods described above, a series of 2β-alkylated gibberellins were prepared but the synthetic routes did not give reliable access to 2α-alkylated gibberellins. These were prepared as follows.

Treatment of GA_3 methyl ester (**50**) with a copper catalysed Grignard reagent and HMPA (scheme 4.16) gave a mixture of both 2α and 2β alkyl GA_1 derivatives (**71**), the ratio of which depended upon the alkyl group being added (table 4.4)[49]. The modified Grignard compound was bulky in comparison with a Gilman organometallic reagent due to the HMPA complexed to the magnesium, and steric interactions between the Grignard reagent and 3β-OH hindered supra-facial attack. Treatment of (**71**) by previously described methods[45] gave the de-esterified gibberellic acids (**72**).

Direct preparation of 2-alkyl GA_4 derivatives was only achieved with lithium dimethylcuprate, the use of Gilman reagents with more bulky alkyl groups caused elimination of the 3-hydroxyl to form a 2,1-(10)-diene; preparation of other 2-alkyl-13-dehydro gibberellins was possible by bridgehead deoxygenation of the appropriate 13-hydroxy GA *via* radical reduction of the oxalate ester[36].



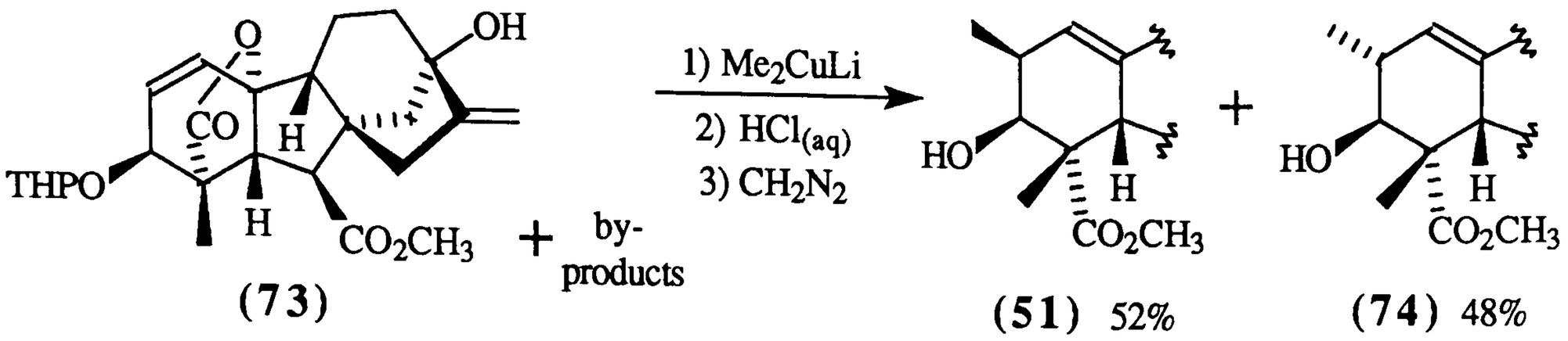
(50) **(71)** **(72)**

Scheme 4.16: Reaction of GA_3 methyl ester with copper catalysed Grignard reagents.

R group	Overall yield of (**71**)	Ratio of α : β
Methyl	70%	3:1
Ethyl	47%	≈1:1
Propyl	41%	3:1

Table 4.4: Results of copper catalysed Grignard reagents addition to (**50**)[36,45].

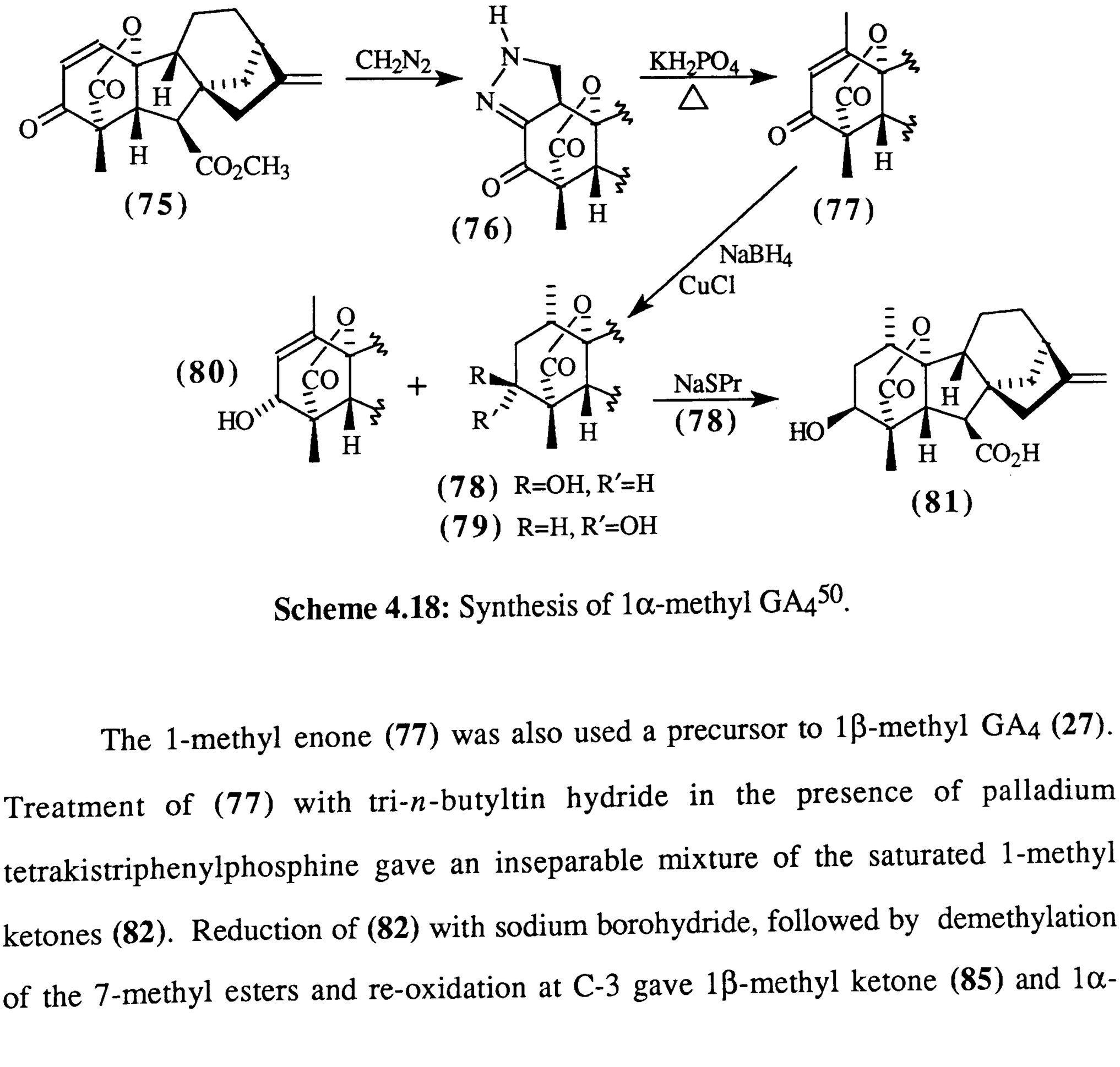
Work by Beale using lithium dimethylcuprate upon the 3-tetrahydropyranyl ether (**73**) gave 100% yield of isomers (**51**) and (**74**) (scheme 4.17). This was due to the large THP group preventing the methyl 'anion' approaching the upper side of ring A, and thus enforcing *syn* addition. As found with the analogous reactions upon the free 3-hydroxyl ester (**50**), use of higher order cuprates gave worse results[47].



(73) (51) 52% (74) 48%

Scheme 4.17: Reaction of **(73)** with lithium dimethyl cuprate.

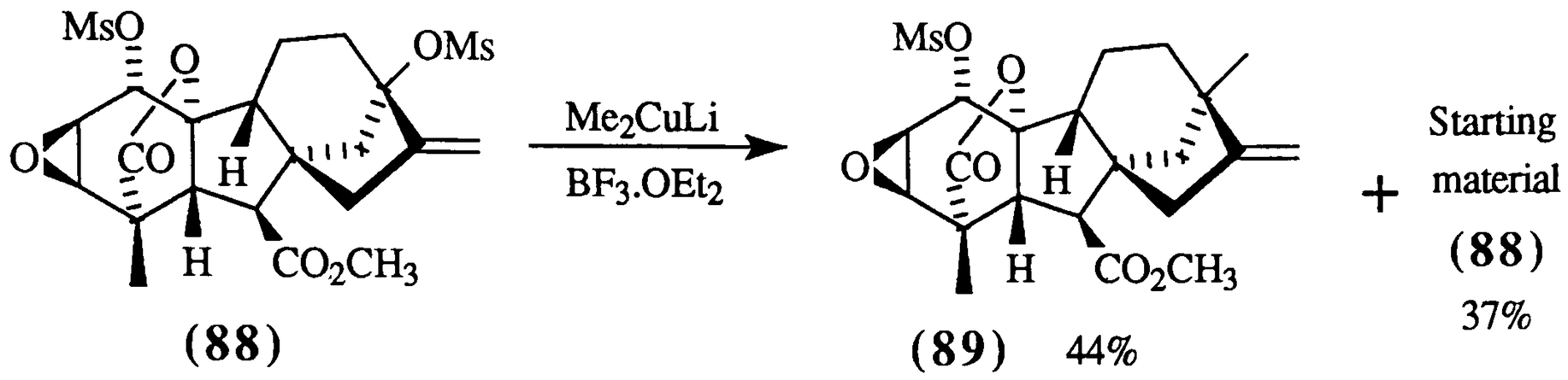
Methylation at the C-1 position was achieved by MacMillan and Willis[50], by treating the enone **(75)** with diazomethane to form the pyrazoline **(76)** and thermolysis gave the β-methyl-α,β-unsaturated ketone **(77)**. Ketone **(77)** was reduced with sodium borohydride and cuprous chloride (to facilitate 1,4 addition of hydride) to give both 1α-methyl GA_4 and 1α-methyl-3-*epi*- GA_4 methyl esters **(78)** and **(79)** respectively, along with some of the 1,2- reduction product **(80)** (scheme 4.18). Treatment of **(78)** with sodium *n*-propylthiolate gave 1α-methyl-GA_4 **(81)**.



Scheme 4.18: Synthesis of 1α-methyl GA_4[50].

The 1-methyl enone **(77)** was also used a precursor to 1β-methyl GA_4 **(27)**. Treatment of **(77)** with tri-*n*-butyltin hydride in the presence of palladium tetrakistriphenylphosphine gave an inseparable mixture of the saturated 1-methyl ketones **(82)**. Reduction of **(82)** with sodium borohydride, followed by demethylation of the 7-methyl esters and re-oxidation at C-3 gave 1β-methyl ketone **(85)** and 1α-

However the mesylate (or indeed the triflate) proved remarkably stable - no reaction occurred under a range of conditions[52]. Interestingly, displacement of a 13-mesylate in **(88)** occurred readily with Gilman reagents to produce **(89)**[53], but no reaction was apparent in ring A upon either epoxides (scheme 4.21) or 3β-acetates[52].

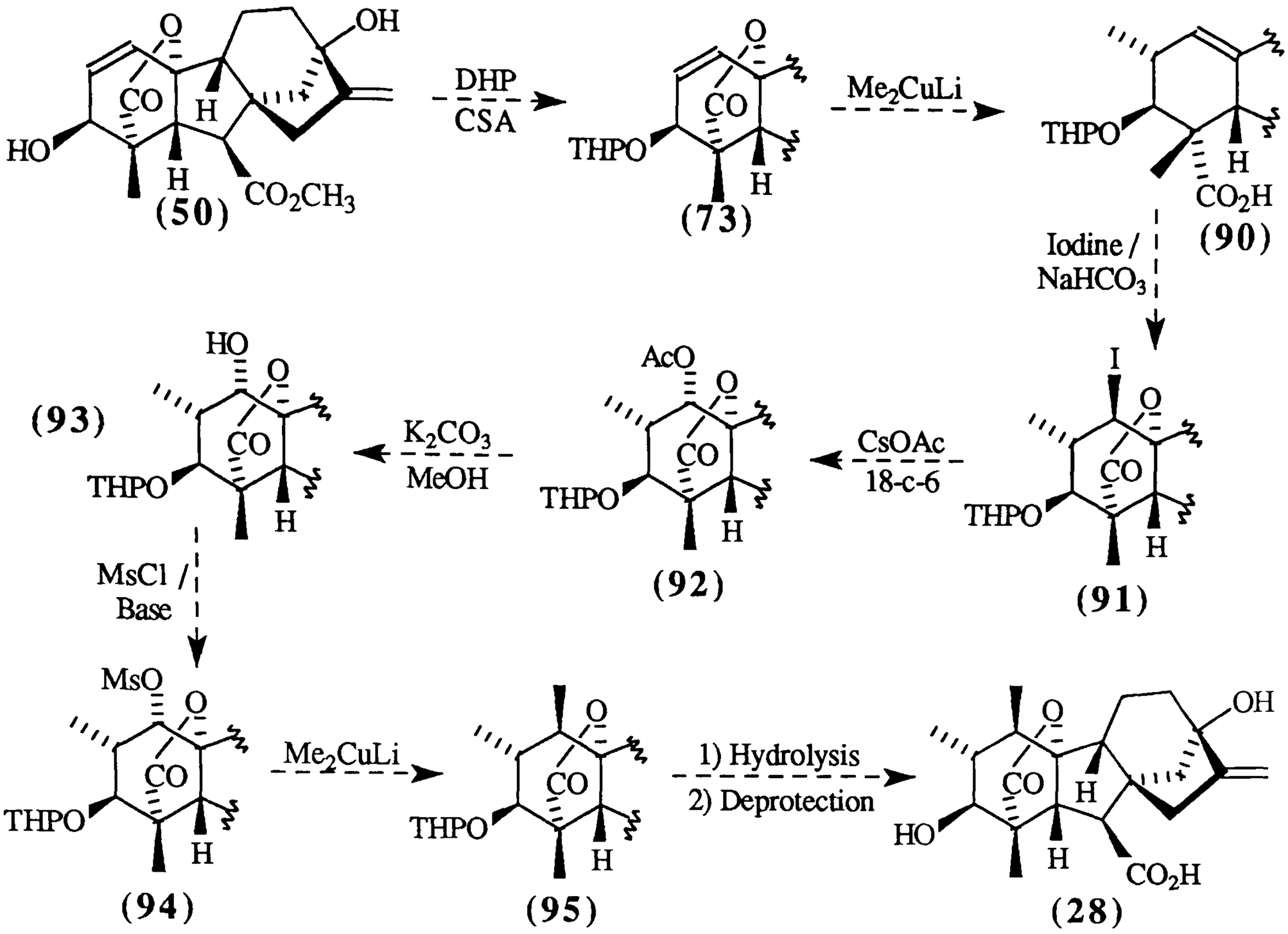


(88) **(89)** 44%

Scheme 4.21: Tertiary mesylate displacement by lithium dimethylcuprate[53].

Thus it was apparent that a new approach to the synthesis of 1β,2α-dimethyl gibberellins was required. The proposed route is shown in scheme 4.22. It is known from previous work that a 2α-methyl group may be introduced via a *syn* S_N2' displacement of the allylic lactone moiety of the 3-tetrahydropyranyl ether of GA_3 methyl ester **(73)**[49].

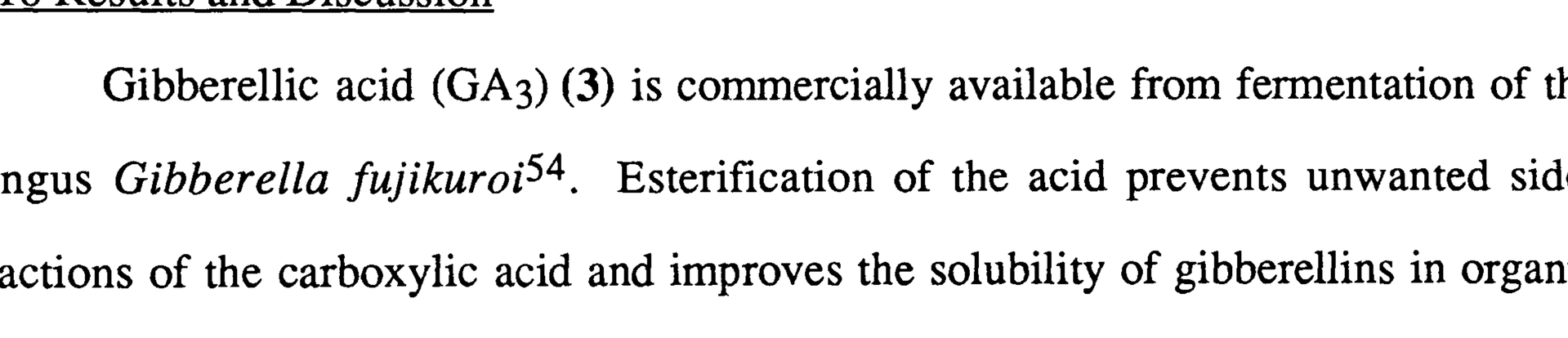
It is proposed that the 1β-methyl group is then introduced *via* displacement of a suitable leaving group at the 1α-position. This leaving group will be introduced *via* a Corey-type iodolactonisation[46] to reform the γ-lactone **(91)**; S_N2 attack upon the axial iodide by an acetate (with cation counter-ion enslaved as a spectator by use of 18-c-6) would give the 1-equatorial ester **(92)**, which would be de-esterified by aqueous potassium carbonate to give an alcohol **(93)**. Use of methanesulfonyl chloride with base should react only with the secondary alcohol formed, and not the bridgehead hydroxyl at C-13, to form a 1α-sulfonate ester **(94)**, which a second Gilman agent should displace to form the required A ring disubstitution **(95)**; deprotection of the 3β-alcohol and de-esterification would form the acid **(28)** for bioassay. Removal of the 3-hydroxyl protecting group prior to the second cuprate reaction's axial attack at C-1 may be necessary for steric reasons.



Scheme 4.22: Proposed route to 1β,2α-dimethyl GA_1 **(28)**.

4.10 Results and Discussion

Gibberellic acid (GA_3) **(3)** is commercially available from fermentation of the fungus *Gibberella fujikuroi*[54]. Esterification of the acid prevents unwanted side-reactions of the carboxylic acid and improves the solubility of gibberellins in organic solvents; for example GA_3 is insoluble in chloroform, whereas the methyl ester **(50)** is sparingly soluble. Treatment of an available mixture of **(3)** and **(50)** with caesium fluoride and methyl iodide according to the method of Otera *et al*[55] gave the required ester **(50)** in approximately 92% yield (scheme 4.23). All subsequent reactions were upon 7-methyl esters.

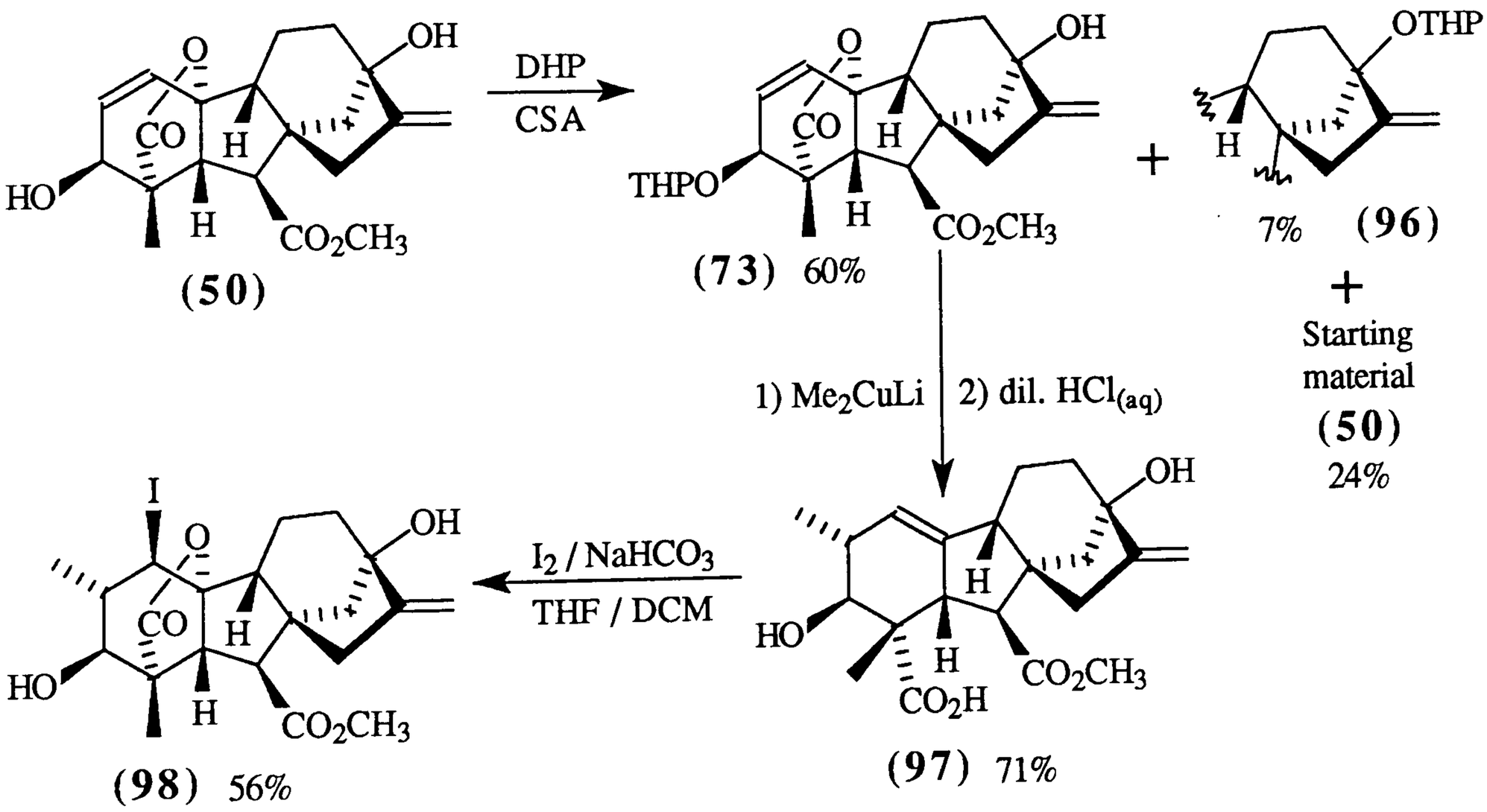


Scheme 4.23: Methylation of 7-carboxylic acid.

The 3-THP ether (**73**) was prepared in 60% yield by treatment of GA_3 methyl ester (**50**) with dihydropyran in the presence of CSA followed by purification of the reaction mixture by flash chromatography. In addition, the 3,13-diTHP derivative (**96**) (7%) and unreacted starting material (24%) were recovered; the by-product (**96**) could be recycled to (**50**) quantitatively by stirring in dilute hydrochloric acid, extracting and removing solvent; no further purification was necessary (scheme 4.24).

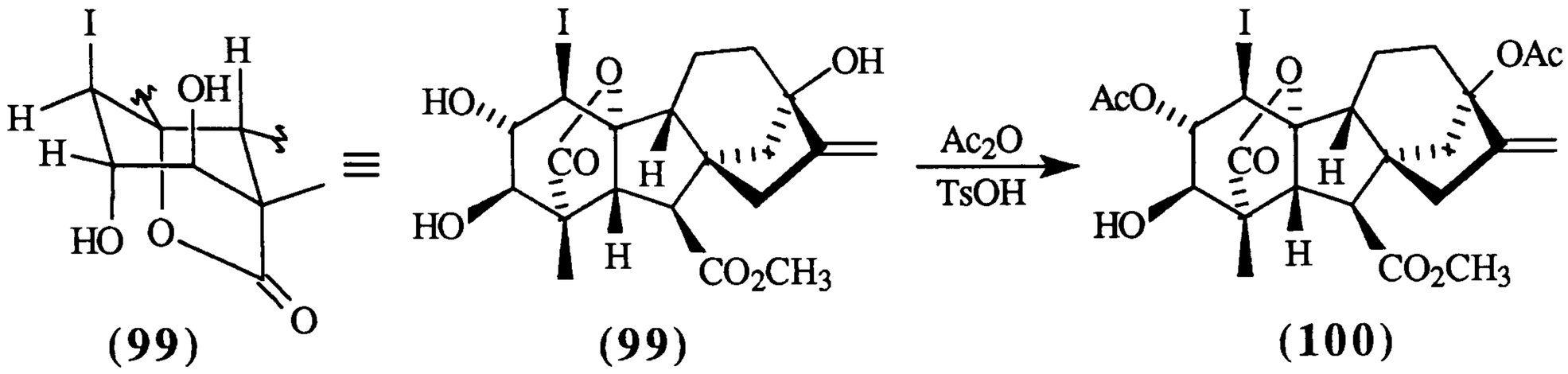
The next stage of the synthesis of 1β,2α-dimethyl GA_1 (**29**) required the introduction of the 2α-methyl group *via* a *syn* S_N2' displacement of the allylic lactone moiety. Treatment of (**73**) with lithium dimethylcuprate gave (**97**) alone, none of the 2β-methyl derivative was isolated, in contrast with work by both Fowles using copper catalysed Grignard reagents[49] and Beale using lithium dimethylcuprate[47] (schemes 4.16 and 4.17).

As scheme 4.24 indicates, the yield of the 2α-alkyl product, (**97**), was higher than those previously obtained, this may be due to the fact that pure starting material (**73**) was used. Both Beale[47] and Fowles[49] had previously used the crude mixture from the THP reaction and the by-products may have interfered with the cuprate addition. This hypothesis is supported by the results of reacting the 3,13-di-OTHP (**96**) with lithium dimethylcuprate which failed to form any identifiable products.



Scheme 4.24: Preparation of iodide (**98**).

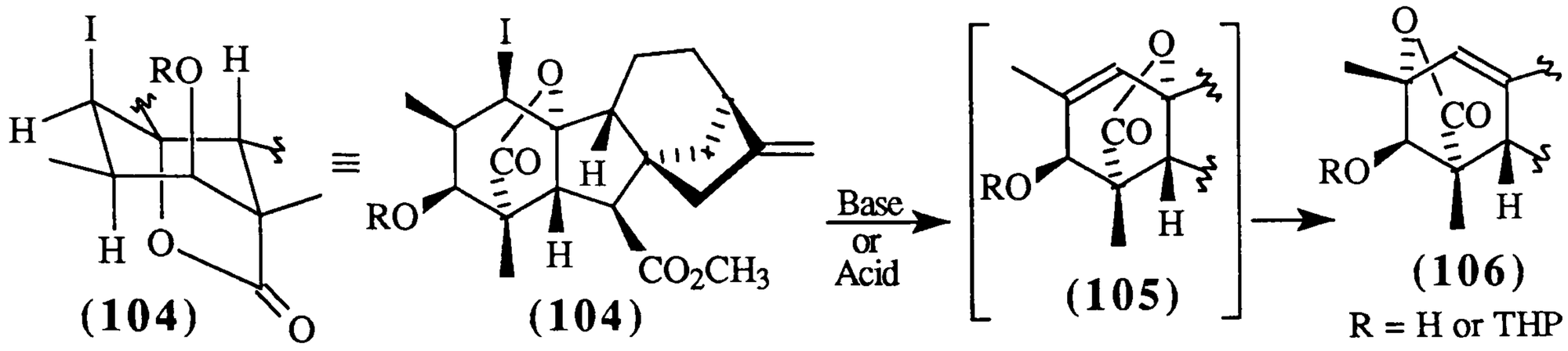
In the proposed synthetic route to the target dimethylated gibberellin (**28**) (Scheme 4.22), it was necessary to retain a protecting group on the 3β-alcohol almost to the final stage of the synthesis. However, it was found that during the work-up of the cuprate reaction mixture under acidic conditions, the acetal was repeatedly removed (as had been noted previously in this reaction[36,47]). Only on one occasion was the protected derivative (**90**) isolated, but in a disappointing 10% yield. Thus it would be necessary to re-protect the 3β-alcohol at some stage prior to the introduction of a hydroxyl group at C-1, or two secondary alcohols might present difficulty in selective sulfonate esterification.



Scheme 4.25: Selective acetylation of triol[56].

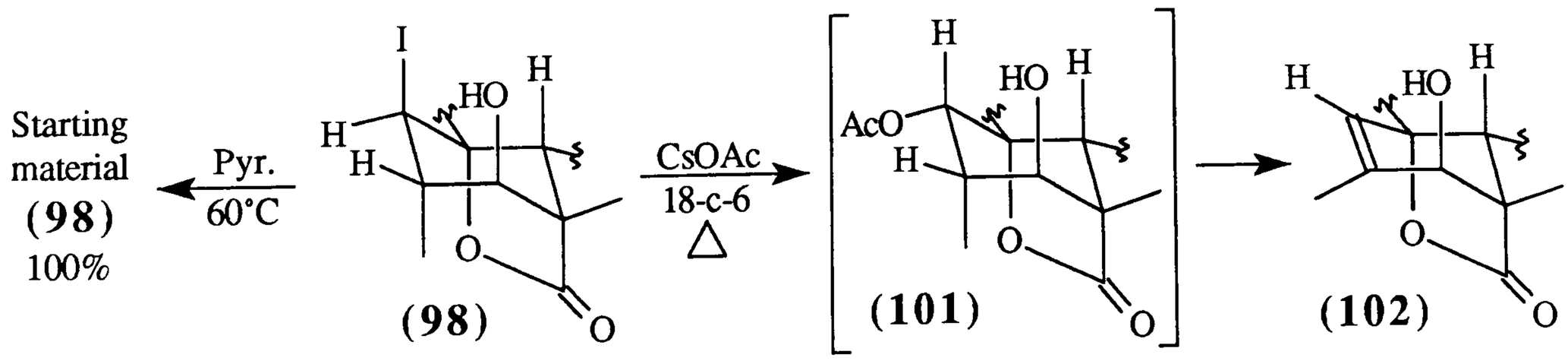
The 19,10γ-lactone, characteristic of the C_{19}-gibberellins, was first regenerated under standard iodolactonisation conditions[46,49] giving (**98**) in 56% yield (scheme 4.24). Attempted protection of the 3β-alcohol of iodolactone (**98**) with a THP group failed; this was most probably due to the steric congestion caused by the transannular interaction between the 1β-iodide and the 3β-alcohol in (**98**). It has been previously shown that the 2α-alcohol of the 1β-iodo-2α,3β-diol (**99**) may be selectively protected as the acetate as a result of this steric crowding of the 3β-alcohol[56] (scheme 4.25). Hence it was decided to delay protection of the 3-alcohol to later in the synthesis.

6 in toluene to 99°C for 2.5 hours, to determine if a S_N1 mechanism was allowing the loss of the iodide; however on extraction, quantitative return of starting material (**98**) was found, suggesting that the acetate anion was in fact the active nucleophile. Interestingly, in previous work by Fowles[36,49] aimed at the preparation of 2-methyl GA_7, neither the unsaturated alcohol or THP derivatives (**105**) could be isolated (scheme 4.27). In both cases rearrangement to the isomeric 1,(10)-ene-19,2-lactones (**106**) occurred. In the case of the 2β-methyl iodide (**104**), a simple E2 mechanism involving *trans*-diaxial elimination of HI was possible with the methyl group in the equatorial position at C-2.



Scheme 4.27: Previous eliminations to a 1,10 lactone[36,49].

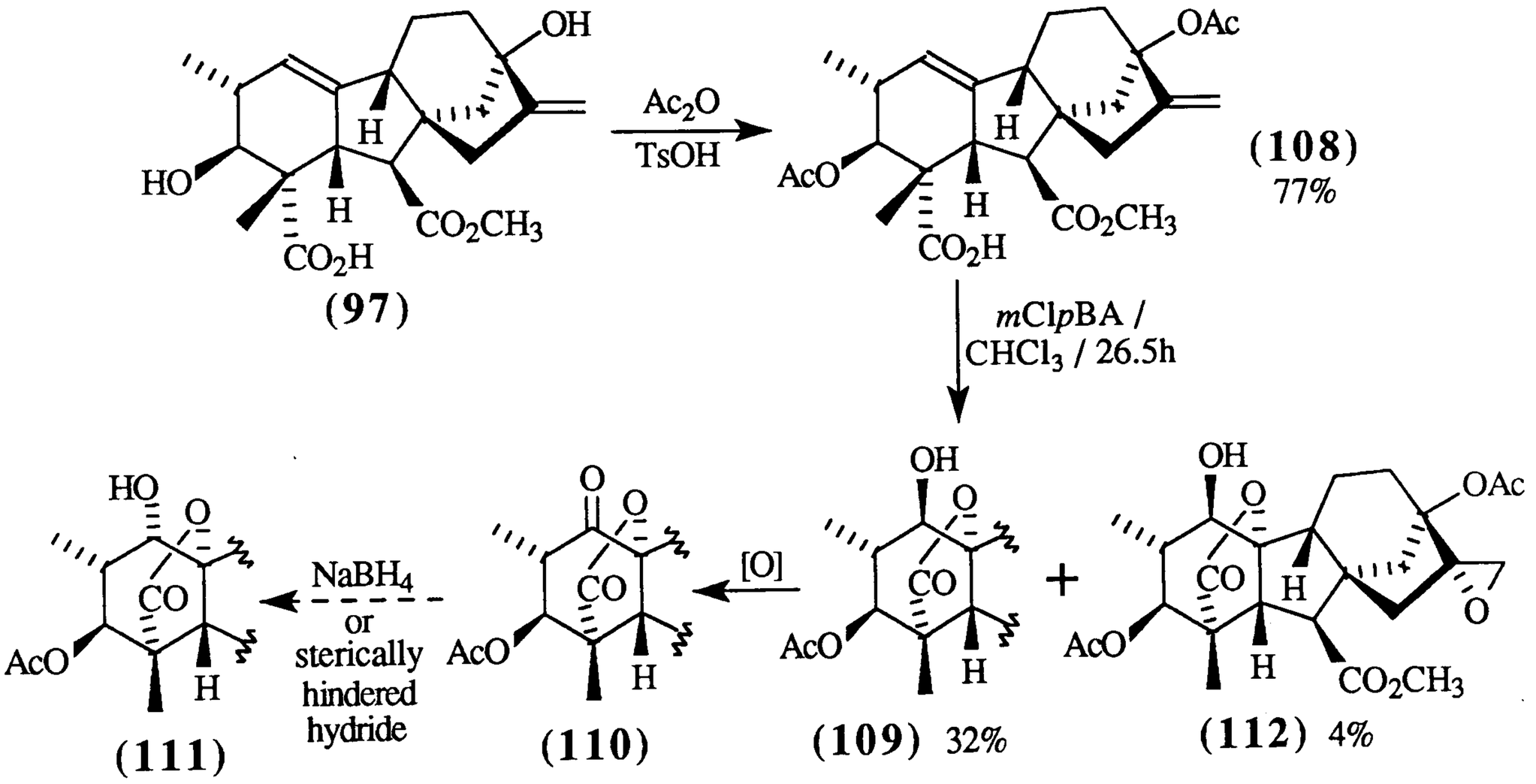
The 1β-I, 2β-H ax.-eq. elimination is so inhibited that the iodolactone (**98**) can be heated in pyridine at 60°C with quantitative return of starting material (scheme 4.28), as the molecule is not aligned for either *syn* or *anti* elimination, but would need the ring to twist and distort to allow elimination. Therefore it would seem that the 1α-acetate, 2α-methyl gibberellin (**101**) is being transiently formed, but then immediately undergoes *syn* elimination.



Scheme 4.28: 3-D structures of products from treatment of (**98**) with alkaline reagents.

An alternative approach for the introduction of a 1α-alcohol was *via* the direct S_N2 displacement of the 1β-iodide of (**98**) with hydroxide (scheme 4.26). Ideally it is

necessary to protect the 3-hydroxyl group prior to reaction with base to prevent epimerisation at C-3 *via* a retro-aldol reaction[35]. However, since the iodolactone (**98**) did not react with DHP and CSA to form the 3β-OTHP protected iodolactone (**91**), (*N. B*. scheme 4.25), a model study was done on the free 3β-hydroxy derivative (**98**), using mildly alkaline conditions. Use of 0.1mM NaOH (with a *p*H 10.2 measured) in a solvent that was 80% v/v water and 20% v/v acetone, returned only starting material in quantitative yield. Repetition of the experiment using 0.5mM NaOH (using the same solvent) gave 36% starting material and 15% 3α-hydroxy iodolactone (**107**) (scheme 4.26). However, the use of 3mM NaOH (in 50:50 v/v water : THF), which was used to assess if the hydroxide was sufficiently active to displace the iodide, gave 84% (**107**) plus 12% starting material. This indicates that the nucleophilic substitution of the iodide with a hydroxide ion is not feasible.

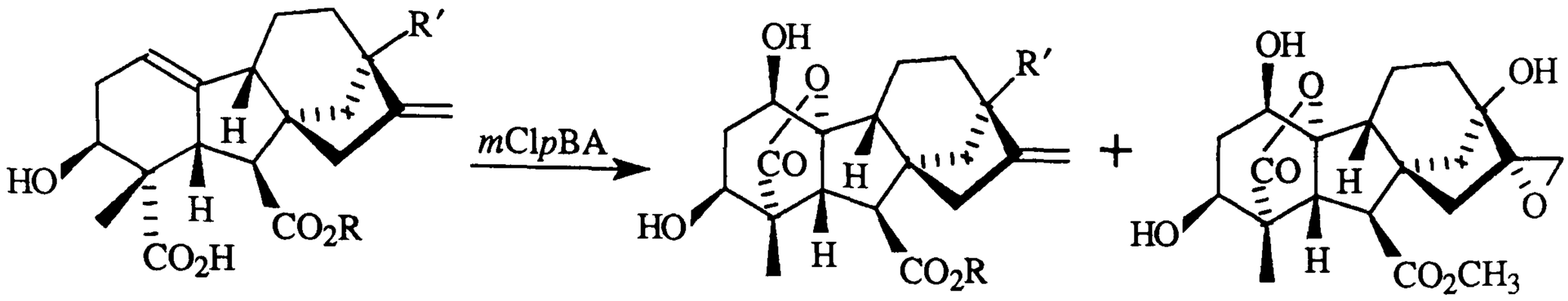


Scheme 4.29: Preparation of ketone (**110**) from (**97**).

Due to the lack of success using an iodolactone to obtain further derivatives, the proposed synthetic route was modified such that an alcohol rather than an iodide was introduced at C-1 during the regeneration of the 19,10γ-lactone (scheme 4.29).

Murofushi *et al*[59] had treated (**113**) and (**114**) with 1 eq. of *m*Cl*p*BA to synthesise GA_{54} (**115**) and GA_{55} (**116**) respectively; however, both lactones were obtained in a yield of only 18% (scheme 4.30). Treatment of the 7-methyl ester (**117**) with 1.6 eq. of *m*Cl*p*BA gave the 19,10-lactone epoxide (**118**) in 14% yield, the oxirane

of which was returned to the olefin (**119**) in 58% yield by use of a modified Cornforth reaction[60]. No other products were identified from the peracid mediated cyclisation reactions[59].



R,R′=H (**113**)
R=H, R′=OH (**114**)
R=CH_3, R′=OH (**117**)

R,R′=H (**115**)
R=H, R′=OH (**116**)
R=CH_3, R′=OH (**119**)

(**118**)

Scheme 4.30: Previous *m*Cl*p*BA induced lactonisation reactions[59].

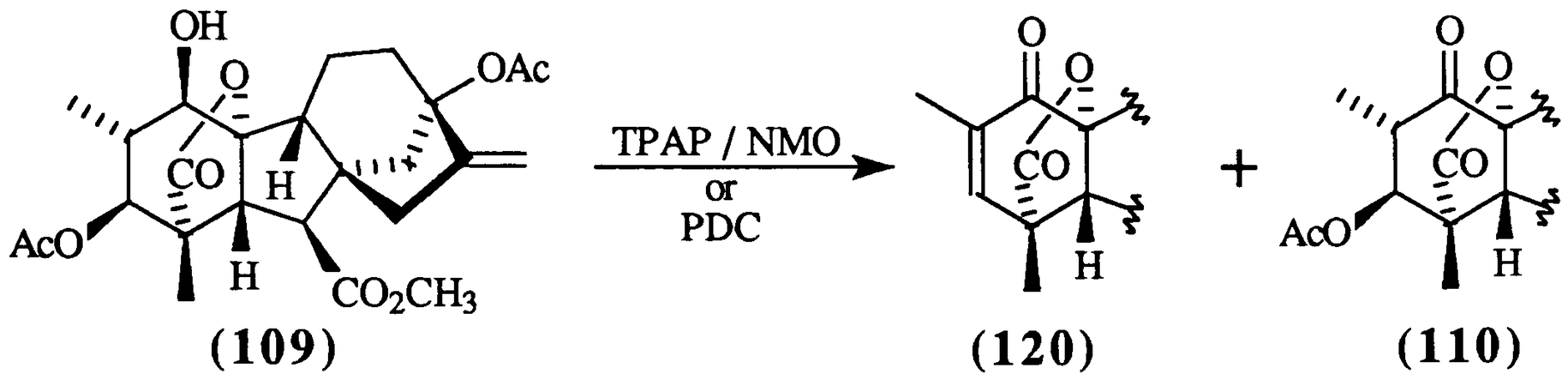
Due to such low yields obtained by Murofushi on cyclisation, (**97**) was treated with acetic anhydride and *p*-toluenesulfonic acid to give the 3,13-diacetate (**108**) in 77% yield (scheme 4.29). This would limit by steric means the approach of the peracid to the exocyclic alkene, and thus avoid the need for a Cornforth reaction.

Treatment of (**108**) (100mg) with *m*Cl*p*BA (0.85eq.) for 26.5 hours gave only 32% of the desired 1β-hydroxy lactone (**109**) as well as 4% of the 16, 17-epoxide lactone (**112**) and 17% starting material. A further mixture of products was obtained which could not be identified by ^{1}H NMR spectroscopy. Repeating the experiment, but using 1.3eq. of *m*Cl*p*BA upon (**108**) for only 4.5 hours gave after purification the required alcohol (**109**) (24%), the epoxide (**112**) (20%), plus a further five impure and unidentified compounds (180mg from 370mg).

Only one isomer of the by-product oxirane (**112**) was found, which was presumed to be the 16α-epoxide, as work by Murofushi[61] using *m*Cl*p*BA upon a GA_5 derivative (scheme 4.2, GA_5 = (**20**)) had produced only the 16α-epoxide, probably due to the 13-hydroxyl directing attack via the less hindered α face of the D ring; however analogous work by Reeve and Crozier upon 2,3-dehydro GA_9[62], the 13-dehydroxy analogue of GA_5, had given both diastereomers of the 16,17-epoxide.

Although the results from this reaction were disappointing, sufficient 1β-alcohol (**109**) was obtained to continue with the synthesis.

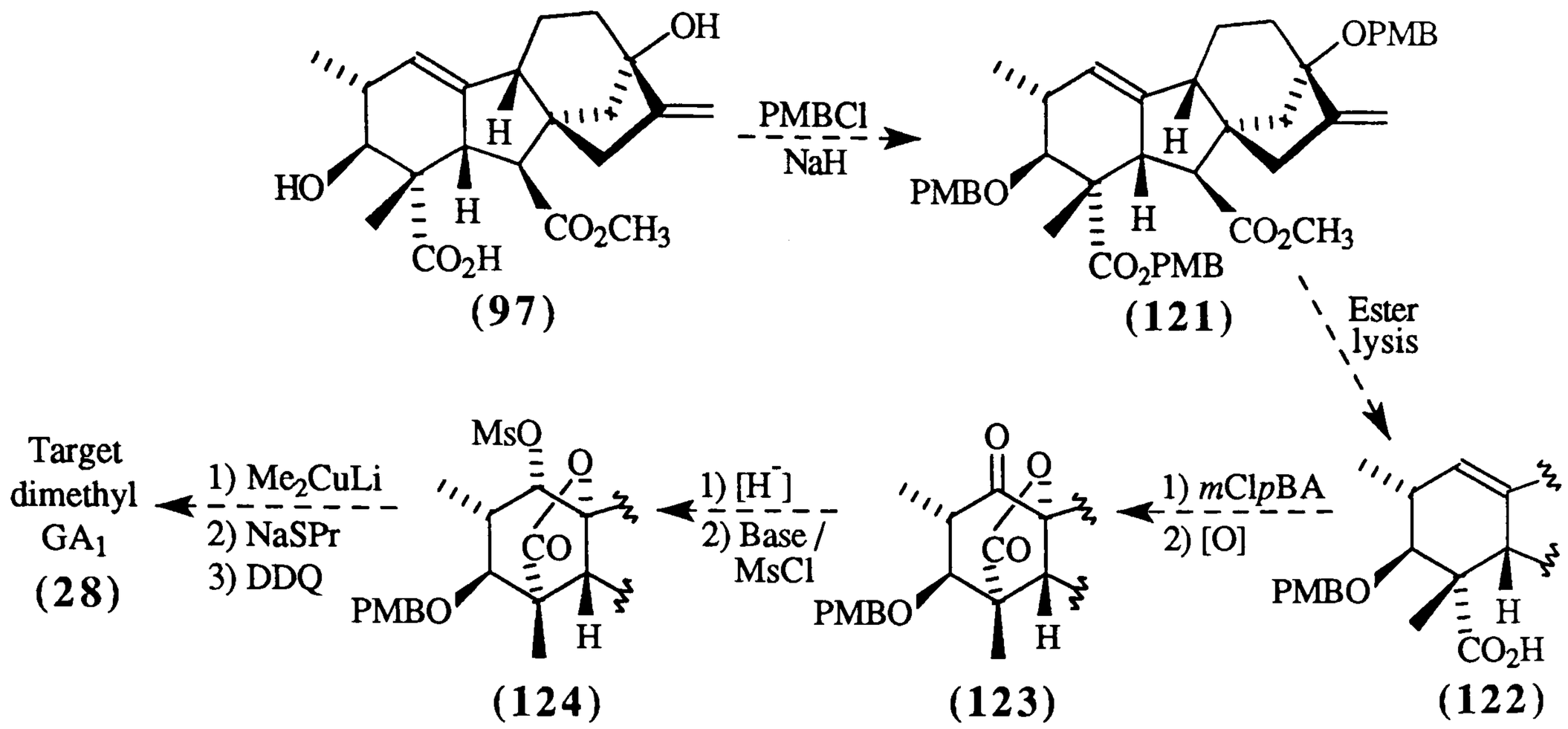
To invert the alcohol at C-1 a two step oxidation/reduction procedure was favoured (scheme 4.29). Oxidation of **(109)** with TPAP and the co-oxidant NMO gave a 1-ketone but was accompanied by elimination of the 3β-acetate giving the α, β-unsaturated ketone **(120)**, as the sole product in 83% yield. The ^{1}H NMR spectrum of **(120)** showed a quartet (J=1.5Hz) at 6.68ppm assigned to 3-H and the ^{13}C NMR spectrum showed signals characteristic of an α,β-unsaturated ketone at 190ppm (C=O), 136ppm (C-2) and 148ppm (C-3). On treatment of the alcohol **(109)** with PDC, an inseparable mixture of **(120)** and the required acetate **(110)** was obtained in the ratio of 2 : 1 respectively (scheme 4.31). This may be due to the difference in basicity between *N*-methylmorpholine (the redox product) and pyridine, the pK_a's of the conjugate acids being 7.67 and 5.25 respectively[63].



(109) **(120)** **(110)**

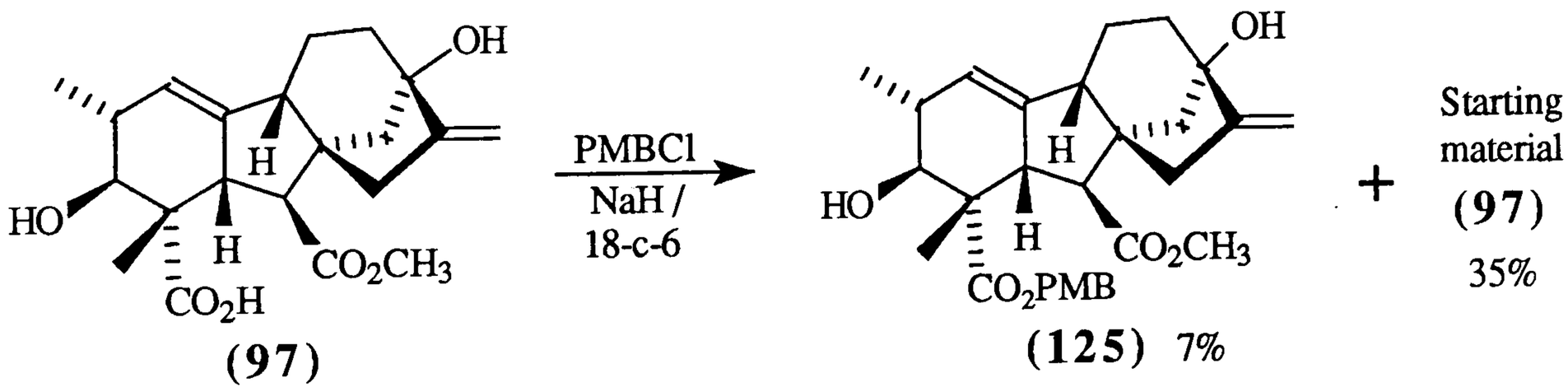
Scheme 4.31: Oxidations of **(109)**.

To prevent elimination during the oxidation, it was apparent that it was necessary to use a protecting group on the 3-alcohol which would give a more poor leaving group, for example an ether. Protection of the 3- and 13-alcohols as the *para*-methoxybenzyl ethers (PMB) **(121)** was proposed, such a group would also add steric bulk to the 13 position, so inhibiting the formation of the exocyclic epoxide during treatment with the peracid, as well as giving a poor leaving group at C-3 (scheme 4.32). Another potential benefit from the use of a bulky and poor leaving group at the 3 position is that it could be put on GA_3 methyl ester **(50)** at the start of the synthesis and used to direct the introduction of the 2α-methyl group in the Gilman reaction.



Scheme 4.32: Proposed use of PMB protection to form **(28)**.

However, the addition of 4.5 equivalents of PMBCl with sodium hydride to acid **(97)** in THF gave no reaction, and heating to reflux simply led to formation of the 19-PMB ester **(125)** in 7% yield. The structure of ester **(125)** was determined from the ^1H NMR spectrum which showed a singlet at 4.94ppm assigned to CO_2-$C\underline{H}_2Ar$, which is more downfield than would be expected due to an PMB ether. In addition 35% of starting material **(97)** was isolated, but none of the required 3-ether **(121)** was apparent (scheme 4.33).



Scheme 4.33: The result from reacting PMBCl with the anion of **(97)**.

Therefore it is apparent that a different protecting group is required at C-3. Unfortunately, further work on this route was not possible due to lack of time.

4.11 Conclusions and Suggestions for Further Work

The synthesis of the 1-oxo, 2α-methyl intermediate (**110**) for the procurement of the target 1β,2α-dimethyl GA_4 (**28**) was achieved in a poor yield of 2.9% from GA_3 in six steps, with several novel compounds and by-products obtained *en route*.

The method of introduction of a 2α-methyl to the gibberellin was improved compared to previous methods, by purification of the 3-THP protected GA_3 methyl ester (**50**). The use of a different 3β-alcohol protecting group, such as a *t*-butyl diphenylsilyl ether, prior to the Gilman reaction may give improved yields by greater steric bulk than a THP acetal. A further advantage is that the protecting group may be less prone to removal under the mildly acidic work-up conditions during work-up, and would facilitate NMR spectra that are more easy to assign as diasteromers would not be formed.

A second problem stage in the synthesis was the per-acid based cyclisation of (**108**) to form (**109**) (scheme 4.29), where a large amount of an epoxide by-product (**112**) was formed and much of the starting material not recovered. Three methods could be considered to improve this reaction:

i) A more bulky group than the acetate could be put on the C-13 of (**97**) such that, on steric grounds, epoxidation of the exocyclic double bond would be less favourable; the 3-*para*-methoxybenzyl ether was not formed, but a silyl group might be added in the presence of a free 19-carboxylic acid.

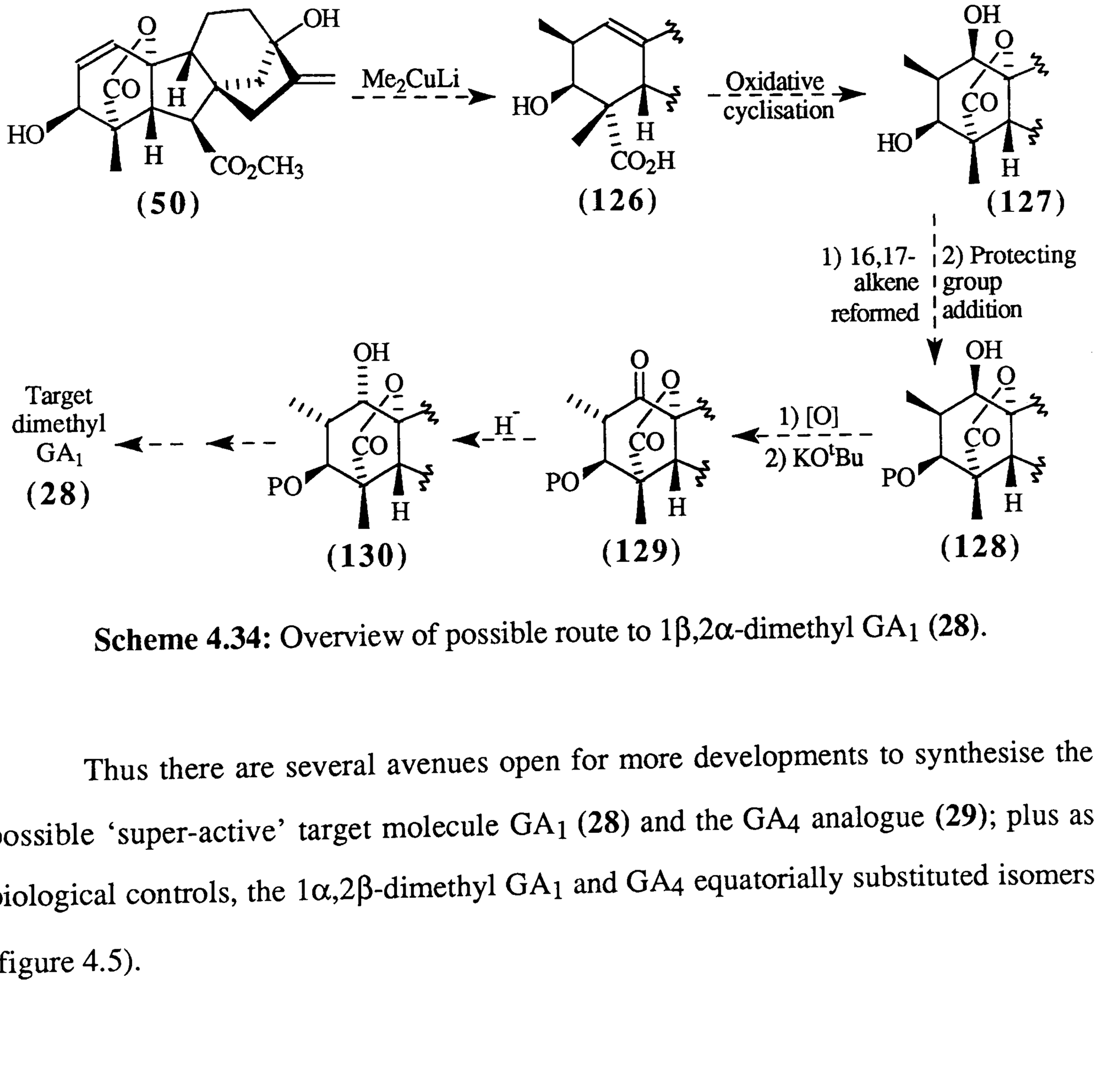
ii) A more stable protecting group could be put on C-3 (and C-13) of (**97**) which would not be cleaved during the reaction.

iii) Addition of an excess of per-acid to enable both double bonds to react, then regeneration of the exocyclic double bond from the 16,17-epoxide by the Cornforth method[60], as has been undertaken previously on gibberellins[59,61,62,64].

Elimination of the acetate in (**110**) to the unwanted α,β-unsaturated ketone (**120**) may be avoided by use of a more mild oxidant than PDC, such as the alkyl cyclochromate (figure 2.3) or silver carbonate on celite[65]. Another possibility is the use of an alcohol dehydrogenase enzyme[66] to form the ketone (**110**); alternatively, a whole cell culture, e.g., *Rhodococcus erythropolis* can invert an alcohol directly[67], so may be

effective upon **(109)**. Similarly an lipase enzyme such as that from *Candida rugosa* (previously named *C. cylindracea*) in phosphate buffer may lyse an ester without any elimination occurring[68].

Formation of the 2β-methyl derivative **(126)** *via* a favoured *anti* S_N2' attack should proceed in higher yield than *syn* addition, which could be cyclized with a peracid **(127)** (scheme 4.34). Addition of a poor leaving group added at the 3 position would prevent elimination when the 1-keto group was formed **(128)**. Nor would the poor leaving group at C-3 eliminate when base was used to invert the 2β-methyl, *via* the enolate and quenching with a bulky proton source to the desired 2α-methyl **(129)**, or when the 1α-alcohol **(130)** was formed by using hydride. The suggested new synthesis may then proceed using a methanesulfonate ester as proposed earlier.



Scheme 4.34: Overview of possible route to 1β,2α-dimethyl GA_1 **(28)**.

Thus there are several avenues open for more developments to synthesise the possible 'super-active' target molecule GA_1 **(28)** and the GA_4 analogue **(29)**; plus as biological controls, the 1α,2β-dimethyl GA_1 and GA_4 equatorially substituted isomers (figure 4.5).

STUDIES TOWARDS THE SYNTHESIS OF 1β,2α-DIMETHYL GIBBERELLINS

CHAPTER FIVE:

Experimental and References

5 Experimental Details and References

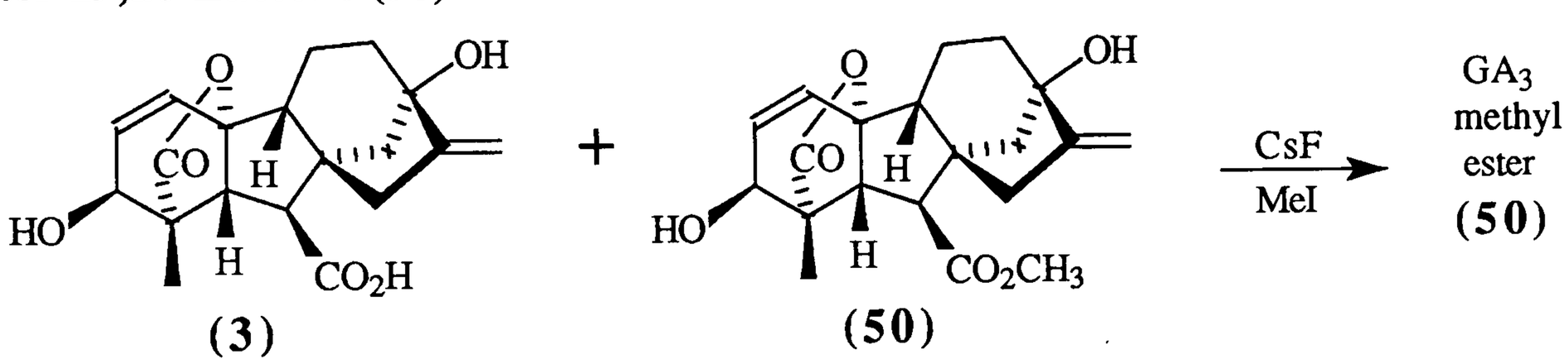
5.1 Standard Work-up Procedure for Gibberellin Products

The methods were the same as for the intermediates towards the mycinolide (section 3.1), but using an acid rather than alkaline work-up media: dilute hydrochloric acid was added until approximately *p*H 3 had been obtained, then extracted with organic solvents. Cuprous iodide was recrystallised[69] and stored at ≈125°C and ≈25mmHg until immediately prior to use. The sole UV spectrum was obtained from a Shimadzu UV160® using 1cm path length quartz cells.

Any deviations from these procedures were noted.

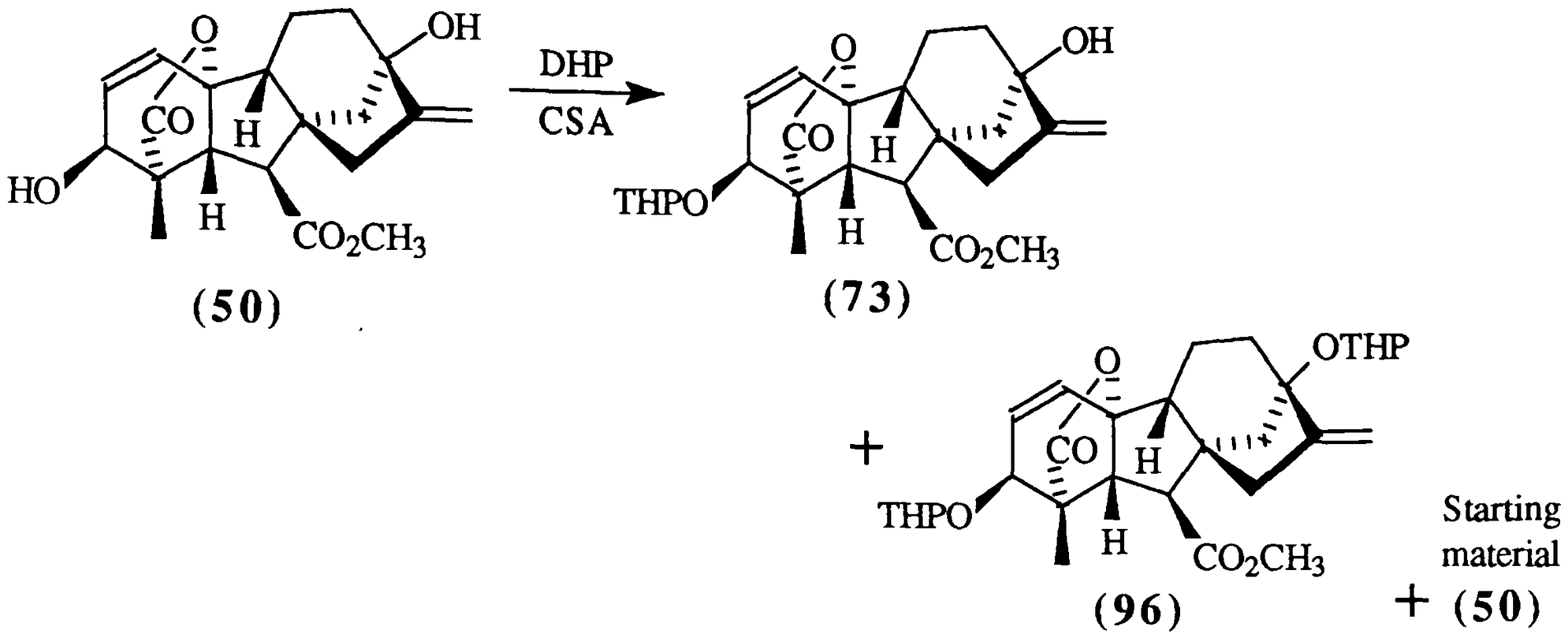
5.2 Experimental

ent-3α,10β,13-Trihydroxy-20-norgibberella-1,16-diene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (50)



To a mixture of GA_3 methyl ester **(50)** and GA_3 **(3)** in approximately 2:1 ratio (6.01g, 11.32 and 5.66mmol respectively) in DMF (50ml) were added caesium fluoride (2.13g, 14.0mmol) and methyl iodide (1.3ml, 14.0mmol), and stirred under anhydrous and light-free conditions for 47h. The reaction mixture was then diluted with brine, and worked-up as usual. The solvents were removed under 0.3mmHg pressure, to give GA_3 methyl ester **(50)** (5.96g, 16.55mmol; -11.32mmol = 5.23mmol, 92%); R_f = 0.68 (100% ethyl acetate); mp 203-206°C (from chloroform and carbon tetrachloride) (lit.[34], 208-210°C); δ_H ($(CD_3)_2CO$) 1.16 (3H, s, 18-H_3), 2.71 (1H, d, *J* 10.5, 6-H), 3.26 (1H, d, *J* 10.5, 5-H), 3.71 (3H, s, OCH_3), 3.83 (1H, br s, OH), 4.06 (1H, m, 3-H), 4.88 (1H, s, 17-H), 5.21 (1H, s, 17-H), 5.89 (1H, dd, *J* 9.5 and 3.5, 2-H), and 6.30 (1H, d, *J* 9.5, 1-H); *m/z* 360 (M^+, 20%), 342 (15), 328 (26), 316 (23), 298 (58), 255 (41), 238 (70), 209 (44), 155 (43), and 136 (100).

***ent*-10β,13-Dihydroxy-3α-O-tetrahydropyranyl-20-norgibberella-1,16-diene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (73)** and ***ent*-10β-Hydroxy-3α,13-di-O-tetrahydropyranyl-20-norgibberella-1,16-diene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (96)**



(50) **(73)** **(96)**

Starting material + **(50)**

GA_3 methyl ester **(50)** (1.102g, 3.06mmol) in DCM (50ml) was treated with CSA (121mg, 0.5mmol) and 3,4-dihydro-2*H*-pyran (0.35ml, 3.84mmol) under anhydrous conditions at 0°C. This was allowed to attain room temp. for 29h and made to *p*H ≈8 with sodium hydrogen carbonate, and then the crude extract obtained as usual except utilizing an alkaline process instead of an acidic environment. The product was purified by column chromatography eluted with ethyl acetate : petrol (20:80), to give as a pale yellow oil the bis-tetrahydrapyranyl ether **(96)**, (112mg, 0.212mmol, 7%), the two diastereomers in a ratio of 59:41 (measured by ^1H NMR spectra integrals); R_f = 0.84 (ethyl acetate : petrol, 50:50); (Found $[MH]^+$, 529.2798. $C_{30}H_{41}O_8$ requires MH, 529.2801); υ_{max}/cm^{-1} 1074 and 1123 (O-C-O) and 1736 and 1777 (C=O). Major isomer, δ_H 1.18 (3H, s, 18-H_3), 2.78 (1H, d, *J* 11, 6-H), 3.32 (1H, d, *J* 11, 5-H), 3.73 (3H, s, OCH_3), 4.01 (1H, d, *J* 3.5, 3-H), 4.64 (1H, m, O-CH-O), 4.79 (1H, m, O-CH-O), 4.99 (1H, s, 17-H), 5.11 (1H, s, 17-H), 6.00 (1H, dd, *J* 9.5 and 3, 2-H) and 6.25 (1H, d, *J* 9.5, 1-H); δ_C 14.41 (C-18), 16.67 (C-11), 19.80, 20.11, 25.24, 25.40, 30.53 and 31.68 (6 x THP: CH_2), 38.37, 40.94 and 43.78 (C-12, C-14, C-15), 49.94 and 53.45 (C-4, C-8), 50.89, 51.05, 51.98 and 53.11 (C-5, C-6, C-9, OCH_3), 62.73 and 63.05 (2 x THP: CH_2O), 76.66 (C-3), 83.43 (C-13), 90.64 (C-10), 94.69 and 101.94 (O-CH-O), 108.04 (C-17), 131.71 and 132.72 (C-1, C-2), 153.96 (C-16), 172.52 (C-7) and 178.52 (C-19). Minor isomer, δ_H 1.28 (3H, s, 18-H_3), 2.76 (1H, d, *J* 11, 6-H), 3.32 (1H, d, *J* 11, 5-H),

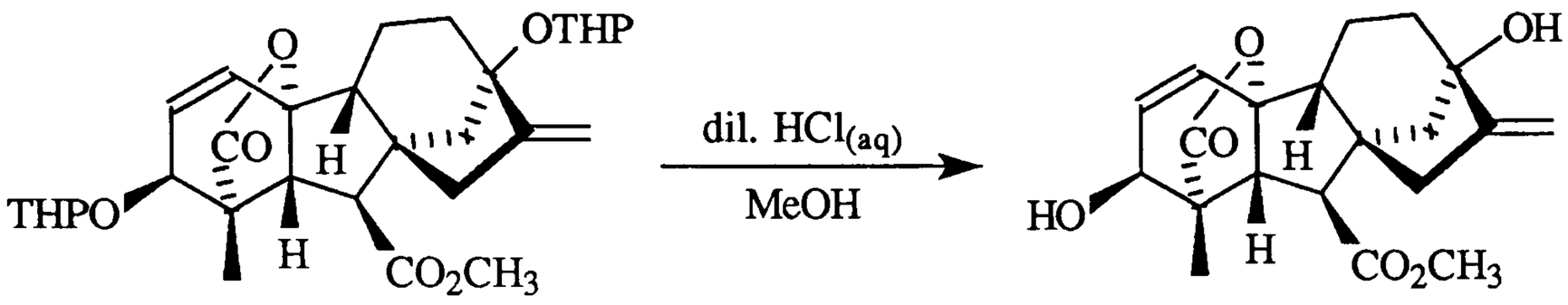
3.73 (3H, s, OCH_3), 4.19 (1H, d, *J* 3.5, 3-H), 4.57 (1H, m, O-CH-O), 4.64 (1H, m, O-CH-O), 4.96 (1H, s, 17-H), 5.26 (1H, s, 17-H), 6.00 (1H, dd, *J* 9.5 and 3, 2-H) and 6.33 (1H, d, *J* 9.5, 1-H); δ_C 14.81 (C-18), 16.91 (C-11), 19.26, 20.11, 25.29, 25.40, 30.62 and 31.69 (6 x THP: CH_2), 36.37, 41.57 and 44.42 (C-12, C-14, C-15), 49.15 and 53.26 (C-4, C-8), 50.72, 51.38, 51.86 and 53.00 (C-5, C-6, C-9, OCH_3), 62.51 and 62.73 (2 x THP: CH_2O), 70.70 (C-3), 82.40 (C-13), 90.70 (C-10), 95.40 and 101.94 (O-CH-O), 108.32 (C-17), 129.66 and 133.17 (C-1, C-2), 154.60 (C-16), 172.58 (C-7) and 178.52 (C-19). *m/z* (CI) 529 ($[MH]^+$, 3%), 473 (3), 459 (1), 445 (21), 427 (15), 361 (16), 343 (67), 281 (75), 239 (93), 103 (15) and 85 (100).

Further elution with ethyl acetate : petrol (30:70) gave the desired 3-O protected alcohol, a crisp white bubbly solid (**73**), (814mg, 1.83mmol, 60%), of two diastereomers in a ratio of 67:33 (measured by ^1H NMR spectra integrals); mp 99-101°C (from chloroform and petrol) (lit., product not fully characterised previously[47]); R_f = 0.48 (ethyl acetate : petrol, 50:50); (Found C, 67.5; H, 7.4%; $[MH]^+$, 445.2214. $C_{25}H_{32}O_7$ requires C, 67.6; H, 7.2%; MH, 445.2226). Major isomer, δ_H 1.19 (3H, s, 18-H_3), 2.75 (1H, d, *J* 11, 6-H), 3.33 (1H, d, *J* 11, 5-H), 3.52 (1H, m, 3-H), 3.73 (3H, s, OCH_3), 4.65 (1H, dd, *J* 4.5 and 2.5, O-CH-O), 4.96 (1H, s, 17-H), 5.28 (1H, s, 17-H), 5.99 (1H, dd, *J* 9.5 and 3.5, 2-H) and 6.26 (1H, d, *J* 9.5, 1-H); δ_C (300MHz) 14.47 (C-18), 17.01 (C-11), 19.85, 25.23 and 30.55 (3 x THP: CH_2), 38.26, 43.11 and 44.77 (C-12, C-14, C-15), 50.37 and 53.36 (C-4, C-8), 50.77, 51.04, 52.20 and 53.15 (C-5, C-6, C-9, OCH_3), 63.19 (THP: CH_2O), 76.67 (C-3), 78.24 (C-13), 90.50 (C-10), 102.10 (O-CH-O), 107.54 (C-17), 131.65 and 132.85 (C-1, C-2), 157.14 (C-16), 172.80 (C-7) and 178.91 (C-19). Minor isomer, δ_H 1.28 (3H, s, 18-H_3), 2.78 (1H, d, *J* 11, 6-H), 3.21 (1H, d, *J* 11, 5-H), 3.52 (1H, m, 3-H), 3.74 (3H, s, OCH_3), 4.81 (1H, m, O-CH-O), 4.96 (1H, s, 17-H), 5.28 (1H, s, 17-H), 6.02 (1H, dd, *J* 9.5 and 3.5, 2-H) and 6.34 (1H, d, *J* 9.5, 1-H); δ_C (300MHz) 14.81 (C-18), 17.02 (C-11), 19.23, 25.29 and 30.62 (3 x THP: CH_2), 38.31, 44.81 and 44.83 (C-12, C-14, C-15), 50.09 and 53.49 (C-4, C-8), 50.85, 50.95, 52.08 and 52.81 (C-5, C-6, C-9, OCH_3), 62.54 (THP: CH_2O), 70.69 (C-3), 78.24 (C-13), 95.36 (C-10), 94.41 (O-CH-O), 107.51 (C-17), 129.78 and 133.10 (C-1, C-2),

153.85 (C-16), 172.82 (C-7) and 178.93 (C-19). *m/z* (CI) 445, ([MH]$^+$, 18%), 427 (10), 413 (3), 395 (5), 383 (28), 361 (12), 343 (70), 299 (58), 281 (98) and 239 (100).

Further elution with ethyl acetate : petrol (50:50) gave starting material (**50**), (260mg, 0.722mmol, 24%), a white powder, R_f = 0.21 (ethyl acetate : petrol, 50:50), data as previously reported.

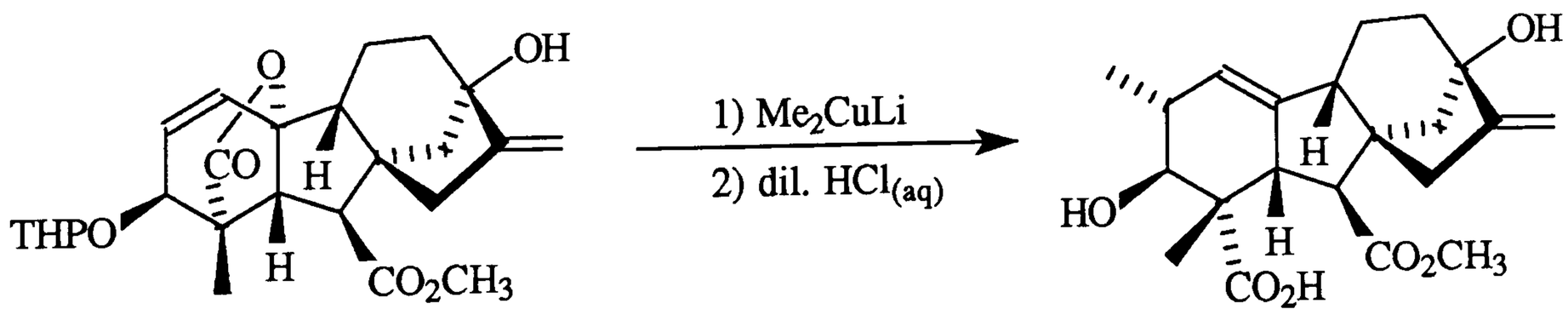
Treatment of (96) with Acid.



(96) **(50)**

The di-protected alcohol (**96**) (112mg, 0.212mmol) was dissolved in methanol (6ml) and 0.001M hydrochloric acid (3ml) added. TLC analysis after 18h indicated no starting material present, so the product was obtained by an alkaline extraction as for the THP protection reaction. The crude product obtained did not require further purification, as it corresponded by both TLC and ^{1}H NMR analysis with GA_3 methyl ester (**50**) (80mg, 0.222mmol, an excess of possible maximum yield).

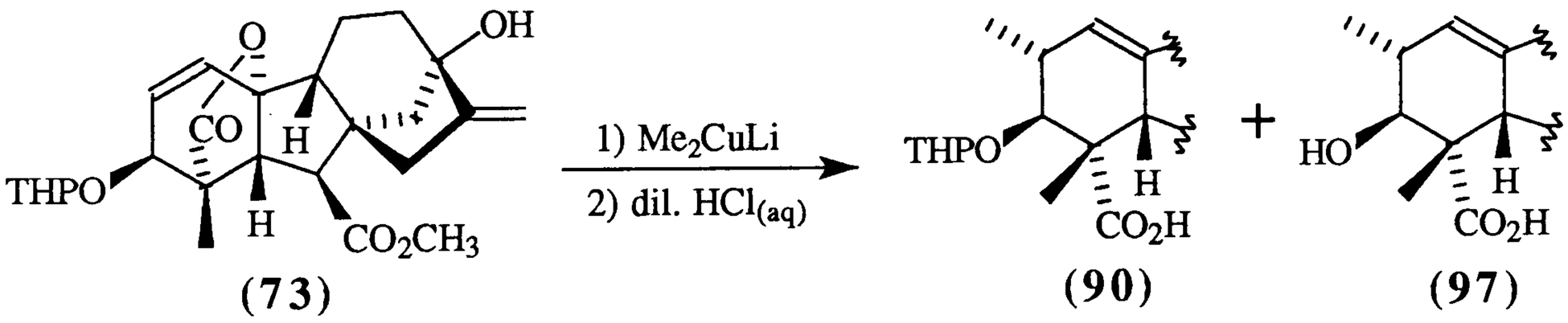
***ent*-3α,13-Dihydroxy-2β-methyl-20-norgibberella-1(10),16-diene-7,19-dioic Acid 7-Methyl Ester (97)** and ***ent*-13-Hydroxy-2β-methyl-3α-O-tetrahydropyran-yl-20-norgibberella-1(10),16-diene-7,19-dioic Acid 7-Methyl Ester (90)**



(73) **(97)**

To a suspension of cuprous iodide (2.51g, 13.2mmol) in ether (20ml) at -10°C was added methyl lithium in ether (17ml, 1.4M, 23.8mmol) over 0.2h until a clear pale

brown solution was formed, and stirred for 0.5h. The dimethyl cuprate solution was added to the THP protected alcohol (**73**) (800mg, 1.80mmol) in ether (20ml) at -78°C over 0.25h, and a deep canary yellow solution formed, which was allowed to reach room temp., and held thus for 2.25h. The temperature was lowered to 0°C and dilute hydrochloric acid added dropwise until *p*H 3-4 was obtained, and left to stir for ≈0.1h. Saturated aqueous sodium hydrogen carbonate was added until *p*H ≈8.5 was reached, and the aqueous system extracted with ethyl acetate (6 x 30ml). The combined organic fractions were separated, and the *p*H lowered with dilute hydrochloric acid (20ml) and stirred for 3.25h, and the normal extraction procedure followed. Following column chromatography, eluting with ethyl acetate : petrol (50:50), a pale cream solid of bubbly appearance was obtained, (**97**) (480mg, 1.28mmol, 71%), mp 95-98°C (from DCM, carbon tetrachloride and petrol) (lit. "a gum"[49]); R_f = 0.44 (100% ethyl acetate); δ_H ($(CD_3)_2CO$) 1.12 (3H, d, *J* 7.5, 2-CH_3), 1.33 (3H, s, 18-H_3), 2.99 (1H, m, 5-H), 3.13 (1H, d, *J* 6, 6-H), 3.70 (3H, s, OCH_3), 3.87 (1H, d, *J* 9.5, 3-H), 4.95 (1H, s, 1-H), 5.00 (1H, s, 17-H) and 5.07 (1H, s, 17-H); *m/z* 376 (M^+, 8%), 358 (33), 340 (6), 326 (16), 316 (17), 283 (14), 254 (14), 253 (100) and 235 (13).



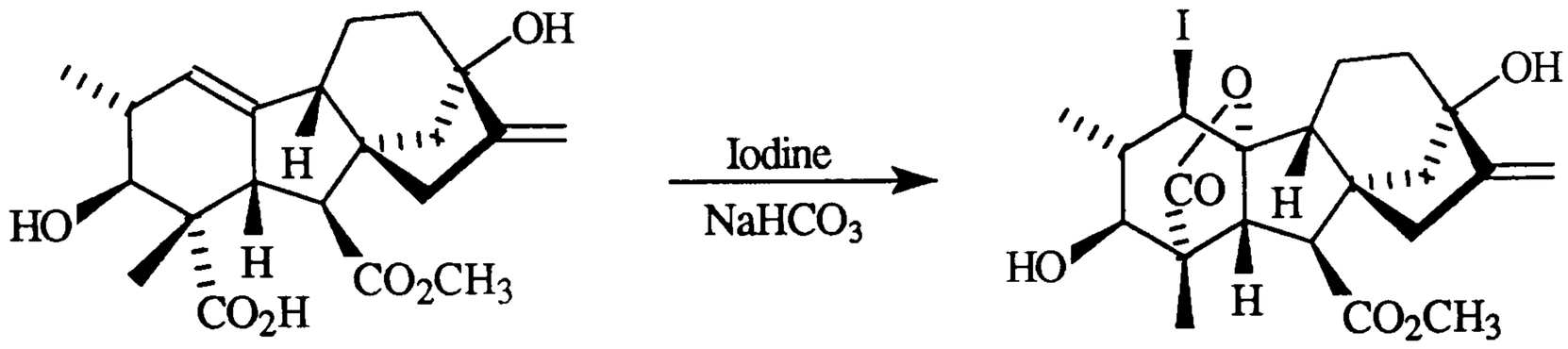
(73) **(90)** **(97)**

Using the previous method of a Gilman reaction upon (**73**) (252mg, 0.670mmol), following work-up and column chromatography, upon one occasion only, the 3-OTHP protected acid (**90**) (54mg, 0.099mmol, 10%) was initially eluted as a colourless and liquid (ethyl acetate : petrol, 30:70). R_f = 0.43 (ethyl acetate : petrol, 50:50); (Found $[MH]^+$, 461.2552. $C_{26}H_{36}O_7$ requires MH, 461.2539); δ_H (300 MHz) major isomer 1.10 (3H, d, *J* 7, 2-CH_3), 1.34 (3H, s, 18-H_3), 3.01 (1H, m, 5-H), 3.48 (1H, d, *J* 6, 6-H), 3.59 (1H, d, *J* 2, 3-H), 3.71 (3H, s, OCH_3) and 4.96 and 4.99 (3H, m, 1-H and 17-H_2); minor isomer 1.12 (3H, d, *J* 7, 2-CH_3), 1.39 (3H, s, 18-H_3), 3.01 (1H, m, 5-H), 3.53* (1H, m, 6-H), 3.56* (1H, m, 3-H), 3.66 (3H, s, OCH_3) and 4.96 and 4.99

(3H, m, 1-H and 17-H_2); *[signals overlap]. *m/z* (CI) 461 ($[MH]^+$, 23%), 443 (7), 407 (30), 389 (36), 377 (29), 375 (26), 359 (60), 327 (63), 299 (91) and 253 (100).

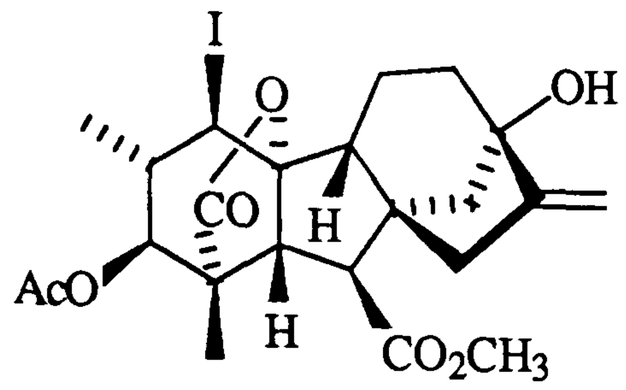
Further elution with ethyl acetate : petrol (50:50) gave the 3-hydroxy acid **(97)** (154mg, 0.307mmol, 34%), with spectral data as obtained previously.

ent-3α-Acetoxy-10β,13-dihydroxy-1α-iodo-2β-methyl-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19,10-Lactone (131) and **_ent_-1α-Iodo-2β-methyl-3α,10β,13-trihydroxy-20-norgibberell-16-ene-7,19-dioic Acid 7-Methyl Ester 19, 10-Lactone (98)**



(97) **(98)**

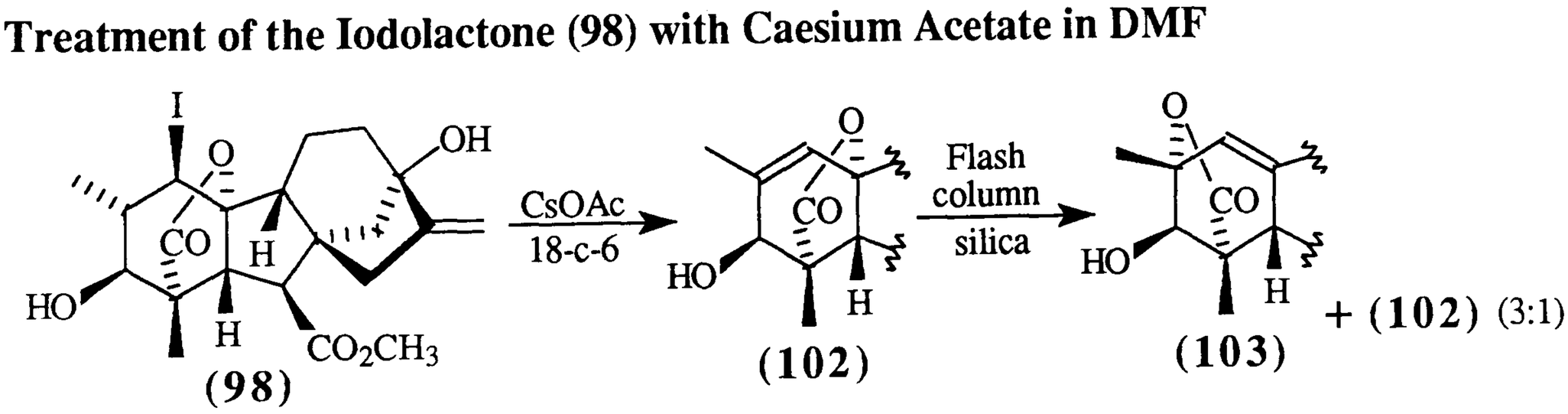
The acid **(97)** (480mg, 1.28mmol) was rapidly stirred in DCM (15ml), THF (12ml), saturated aqueous sodium hydrogen carbonate (7ml) and iodine (379mg, 1.49mmol) at room temp. for 0.9h. Sodium thiosulfate (≈1g) was added, followed by the standard work-up procedures to yield an off-white solid. Subsequent recrystallisation returned white flakes of the iodolactone **(98)** (360mg, 0.717mmol, 56%); R_f = 0.57 (ethyl acetate : petrol, 65:35); mp 181-83°C (from carbon tetrachloride and petrol), (lit. 185°C, [49]); δ_H 1.18 (3H, s, 18-H_3), 1.22 (3H, d, *J* 6.5, 2-CH_3), 2.15 (1H, d, *J* 15.5, 15-H), 2.32 (1H, d, *J* 15.5, 15-H), 2.72* (1H, d, *J* 10, 6-H), 2.75* (1H, t, *J* 9, 9-H), 3.66 (1H, m, 3-H), 3.75 (3H, s, OCH_3), 3.78 (1H, d, *J* 10, 5-H), 4.38 (1H, d, *J* 5, 1-H), 5.00 (1H, br s, 17-H) and 5.28 (1H, br s, 17-H) [* signals overlap]; *m/z* 502 (M^+, 49%), 444 (22), 443 (88), 375 (25), 357 (100), 343 (46), 330 (27), 329 (95), 325 (26) and 253 (90).



(131)

From one such reaction as well as the desired iodolactone **(98)** (154mg, 0.307mmol, 46%) being eluted from a chromatographic column, initial elution with ethyl acetate : petrol (30:70) gave a clear liquid, the 3-O-acetylated product **(131)**, (54mg, 0.0993mmol); R_f = 0.68 (ethyl acetate : petrol, 65:35); (Found M^+, 544.0960. $C_{23}H_{29}O_7I$ requires M, 544.0958); δ_H 1.14 (3H, s, 18-H_3), 1.16 (3H, d, *J* 6.5, 2-CH_3), 2.14 (1H, dt, *J* 15.5 and 2.5, 15-H), 2.32 (1H, d, *J* 15.5, 15-H), 2.66 (1H, d, *J* 10, 6-H), 2.76 (1H, dd, *J* 10.5 and 7, 9-H), 3.74 (3H, s, OCH_3), 3.83 (1H, d, *J* 10, 5-H), 4.42 (1H, d, *J* 5, 1-H), 5.01 (1H, br. s, 17-H), 5.08 (1H, d, *J* 3, 3-H) and 5.29 (1H, dd, *J* 3 and 2, 17-H); δ_C (300 MHz) 14.30 (C-18), 16.97 (C-11), 20.50 and 20.73 (2α-CH_3, $O_2C\underline{C}H_3$), 33.94 and 34.92 (C-1, C-2), 38.07, 42.28 and 45.05 (C-12, C-14, C-15), 49.67* and 53.50 (C-4, C-8), 49.67*, 50.78, 51.38 and 52.19 (C-5, C-6, C-9, OCH_3), 72.95 (C-3), 78.18 (C-13), 95.29 (C-10), 107.84 (C-17), 156.64 (C-16), 170.22 ($O_2\underline{C}CH_3$), 172.24 (C-7) and 176.26 (C-19) [*signals overlap]; *m/z* 544 (M^+, 28%), 502 (8), 485 (16), 418 (12), 417 (43), 385 (9), 358 (25), 357 (79), 325 (48) and 253 (100). As no acetylating agents were used in the process, it was assumed that a small proportion of the starting material had been acetylated previously.

Treatment of the Iodolactone (98) with Caesium Acetate in DMF



(98) **(102)** **(103)** + **(102)** (3:1)

Rapidly crushed caesium acetate (370mg, 1.93mmol) was added to the iodolactone **(98)** (88mg, 0.175mmol) in DMF (20ml), and 18-c-6 (31mg, 0.117mmol)

added. After 2.25h at 70-80°C, the products were extracted in the usual fashion to obtain *ent*-3α,10β,13-trihydroxy-2-methyl-20-norgibberella-1,16-diene-7,19-dioic acid 7-methyl ester 19,10-lactone (**102**) as a brown semi-solid (70mg, 0.187mmol, i.e. excess of possible yield); R_f = 0.47 (ethyl acetate : petrol, 65:35); (Found M^+, 374.1720. $C_{21}H_{26}O_6$ requires M, 374.1729); δ_H 1.23 (3H, s, 18-H_3), 1.82 (3H, d, *J* 1.5, 2-CH_3), 2.76 (1H, d, *J* 11, 6-H), 3.21 (1H, d, *J* 11, 5-H), 3.73 (3H, s, OCH_3), 3.92 (1H, s, 3-H), 4.96 (1H, br s, 17-H), 5.27 (1H, br t, *J* 2.5, 17-H) and 5.99 (1H, d, *J* 1.5, 1-H); δ_C 14.45 (C-18), 17.05 (C-11), 19.46 (2-CH_3), 38.32, 43.11 and 44.76 (C-12, C-14, C-15), 48.80 and 53.91 (C-4, C-8), 50.61, 51.21, 52.10 and 52.92 (C-5, C-6, C-9, OCH_3), 73.46 (C-3), 78.23 (C-13), 91.08 (C-10), 107.53 (C-17), 127.56 (C-1), 140.86 (C-2), 157.06 (C-16), 172.68 (C-7) and 178.61 (C-19); *m/z* 374 (M^+, 10%), 357 (9), 356 (6), 342 (24), 311 (85), 269 (40), 253 (65), 252 (52), 251 (65) and 83 (100).

Purification by column chromatography, eluting with ethyl acetate : petrol (40:60), gave an inseparable mixture of both isomers (**102**) and *ent*-2β,3α,13-trihydroxy-2α-methyl-20-norgibberella-1(10),16-diene-7,19-dioic acid 7-methyl ester 19,2-lactone (**103**) (52mg, 0.139mmol, 79%); analysis by ^1H NMR spectroscopy indicated a ratio of 1:3 respectively; both of R_f = 0.47 (ethyl acetate : petrol, 65:35). Data for the isogibberellin (**103**): δ_H (300 MHz) 1.19 (3H, s, 18-H_3), 1.58 (3H, s, 2β-CH_3), 2.23 (1H, dd, *J* 16.5 and 2.5, 15-H), 2.55 (1H, d, *J* 6, 6-H), 2.61 (1H, m, H-9), 3.21* (1H, d, *J* 6, 5-H), 3.73 (3H, s, OCH_3), 3.90 (1H, s, 3-H), 4.97 (1H, br s, 17-H), 5.12 (1H, br t, *J* 2.5, 17-H) and 5.99 (1H, br s, 1-H) [*signal obscured partially by isomer]; δ_C 17.05 (C-18), 18.67 (C-11), 20.80 (2β-CH_3), 37.02, 38.76 and 48.61 (C-12, C-14, C-15), 45.36 and 53.94 (C-4, C-8), 45.19, 48.80, 49.49 and 52.03 (C-5, C-6, C-9, OCH_3), 78.92 (C-13), 80.42 (C-3), 81.75 (C-2), 106.83 (C-17), 118.48 (C-1), 150.04 (C-10), 153.44 (C-16), 172.25 (C-7) and 176.36 (C-19).

Upon repetition of this experiment with acetonitrile as solvent, upon elution gave again largely a mixture of both isomers, but also a very tiny sample of the isogibberellin (**103**) alone (≈1mg) which gave an extremely weak and unclear ^1H NMR spectra, but was used for MS analysis: (Found M^+, 374.1725. $C_{21}H_{26}O_6$ requires M,

374.1729); *m/z* 374 (M^+, 15%), 357 (11), 356 (8), 343 (17), 342 (31), 312 (35), 311 (100), 269 (47), 253 (79), 252 (59) and 251 (79).

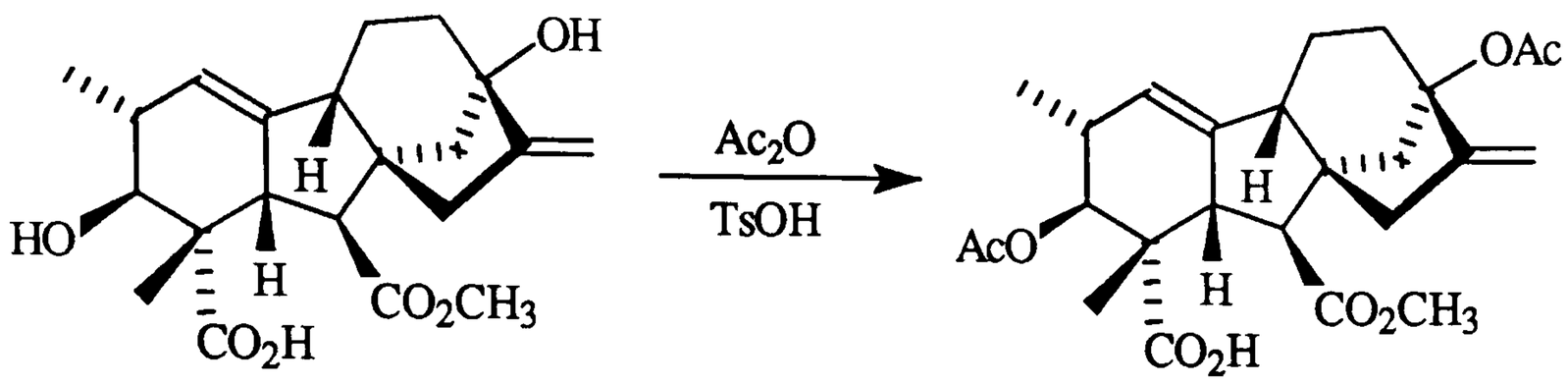
Treatment of the Iodolactone (98) with Sodium Hydroxide.

(98) **(107)** 7 : 1 **(98)**

To the iodolactone **(98)** (25mg, 0.0498mmol) dissolved in THF (10ml) was added, over 0.1h, aqueous sodium hydroxide (10ml, 0.006M, 0.06mmol). By TLC analysis after 3.75h most of the starting material had disappeared, so the crude product was extracted as usual. Purification by column chromatography (using ethyl acetate : petrol, 30:70) gave starting material **(98)** (3mg, 12%).

Further elution with the same solvent system gave *ent*-1α-iodo-2β-methyl-3β,10β,13-trihydroxy-20-norgibberell-16-ene-7,19-dioic acid 7-methyl ester 19, 10-lactone **(107)** (21mg, 0.0418mmol, 84%); R_f = 0.28 (ethyl acetate : petrol, 50:50); mp 153-55°C (from chloroform and petrol); (Found M^+, 502.0838. $C_{21}H_{27}O_6I$ requires M, 502.0852); δ_H 1.18 (3H, s, 18-H_3), 1.25 (3H, d, *J* 6, 2-CH_3), 1.37 (1H, m, 2-H), 2.11 (1H, dt, *J* 15.5 and 3, 15-H), 2.28 (1H, br d, *J* 15.5, 15-H), 2.62 (1H, m, 9-H), 2.73 (1H, d, *J* 10, 6-H), 3.34 (1H, m, 3-H), 3.49 (1H, d, *J* 10, 5-H), 3.74 (3H, s, OCH_3), 4.53 (1H, d, *J* 5, 1-H), 5.00 (1H, s, 17-H) and 5.28 (1H, dd, *J* 3 and 1.5, 17-H); δ_C (300 MHz) 12.68 (C-18), 17.18 (C-11), 21.91 (2α-CH_3), 38.04, 42.28 and 45.26 (C-12, C-14, C-15), 38.95 and 39.73 (C-1, C-2), 50.19 and 54.55 (C-4, C-8), 51.11, 51.29, 52.22 and 54.41 (C-5, C-6, C-9, OCH_3), 75.63 (C-3), 78.19 (C-13), 94.26 (C-10), 107.80 (C-17), 156.54 (C-16), 172.30 (C-7) and 177.03 (C-19); *m/z* 502 (M^+, 42%), 471 (9), 443 (43), 375 (27), 358 (35), 357 (100), 343 (34), 329 (32), 315 (32), and 269 (64).

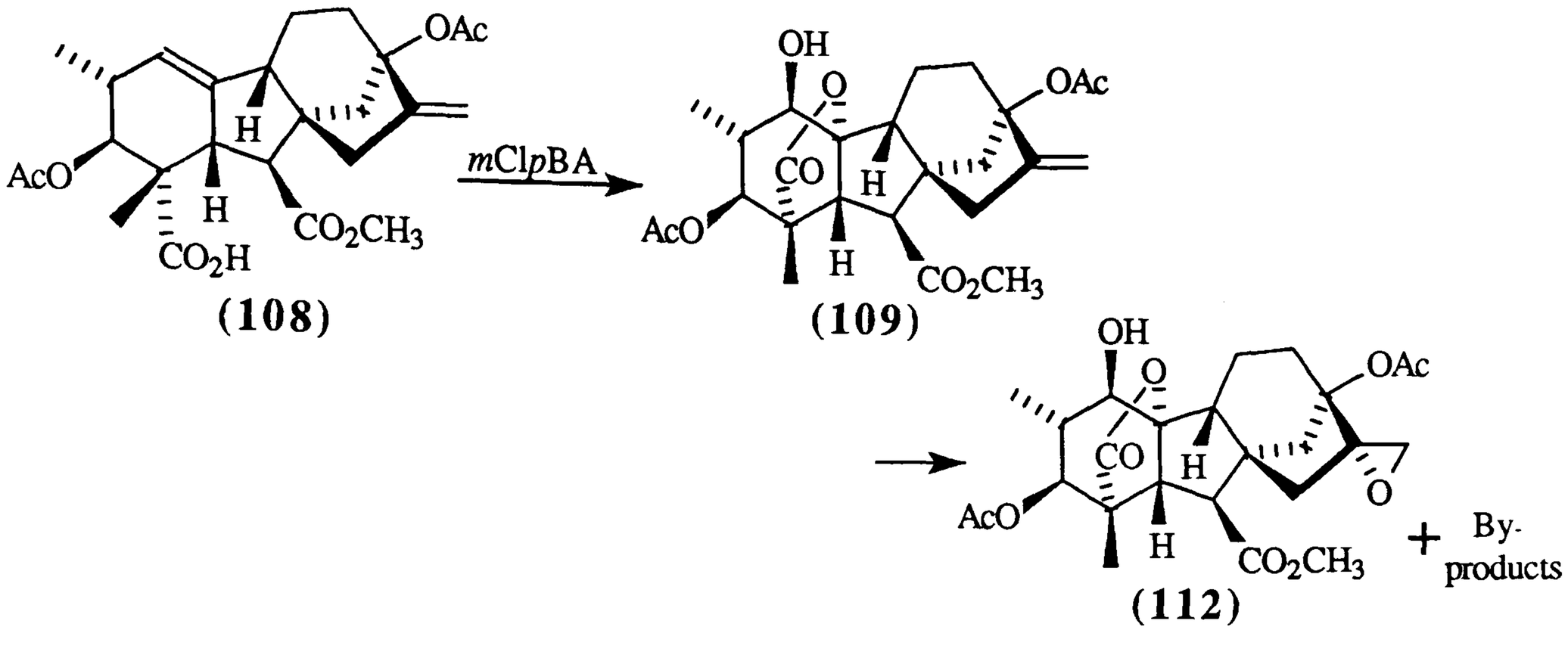
ent-3α,13-Diacetoxy-2β-methyl-20-norgibberella-1(10),16-diene-7,19-dioic Acid 7-Methyl Ester (108)



(97) **(108)**

The acid **(97)** (500mg, 1.33mmol) was added to acetic anhydride (15ml) and tosic acid (19mg) added; the solution was stirred for 17h. By TLC analysis all starting material had disappeared to one spot of $R_f = 0.74$ (ethyl acetate : petrol, 50:50), thus the reaction mixture was diluted with water (30ml) and extracted with chloroform (4 x 30ml). The combined organic layers were extracted with brine (20ml) to which 1 drop conc. sulfuric acid had been added, then separated, the organic portion filtered and evaporated under ≈0.15mmHg vacuum at 40°C. Following column chromatography (eluting with ethyl acetate : petrol, 30:70), the di-acetylated product **(108)** was obtained as white needles (470mg, 1.02mmol, 77%); $R_f = 0.16$ (ethyl acetate : petrol, 30:70); mp 129-132°C (from DCM and petrol); (Found C, 64.9; H, 7.1%; $[MH]^+$, 461.2172. $C_{25}H_{32}O_8$ requires C, 65.2; H, 7.0%; MH, 461.2175); υ_{max}/cm^{-1} 1616 and 1650 (C=C) and 1731 and 1740 (C=O); δ_H (300 MHz) 0.95 (3H, d, *J* 7, 2-CH_3), 1.18 (3H, s, 18-H_3), 2.00 and 2.12 (each 3H, each s, each O_2CCH_3), 2.69 (1H, ap dt, *J* 16 and 3, 9-H), 2.92 (1H, m, 5-H), 3.04 (1H, m, 2-H), 3.25 (1H, d, *J* 6, 6-H), 3.71 (3H, s, OCH_3), 5.00 (2H, s, 1-H and 17-H), 5.07 (1H, s, 17-H) and 5.47 (1H, d, *J* 3.5, 3-H); δ_C 16.38 (C-18), 18.32 (C-11), 20.83, 21.91 and 22.13 (2α-CH_3, 2 x $O_2C\underline{C}H_3$), 32.41 (C-2), 35.46, 38.92 and 44.19 (C-12, C-14, C-15), 46.13, 46.19, 48.73 and 51.69 (C-5, C-6, C-9, OCH_3), 48.57 and 51.34 (C-4, C-8), 75.18 (C-3), 86.29 (C-13), 106.23 (C-17), 116.86 (C-1), 140.64 (C-10), 150.55 (C-16) and 169.95, 170.99, 176.58 and 177.25 (C-7, C-19, 2 x $O_2\underline{C}CH_3$); *m/z* (CI) 461 ($[MH]^+$, 16%), 443 (2), 429 (4), 419 (4), 401 (32), 400 (21), 369 (29), 355 (17), 342 (26) and 341 (100).

Treatment of Diacetate (108) with *meta*Chloro*per*benzoic Acid



(108) **(109)** **(112)**

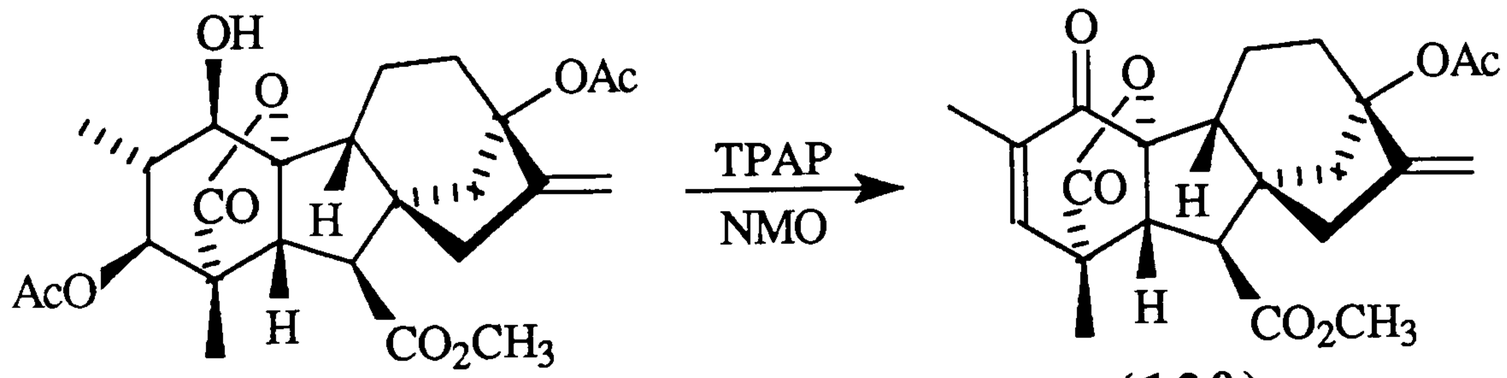
The diacetylated product **(108)** (100mg, 0.217mmol) was dissolved in chloroform at 0°C and *m*Cl*p*BA (40mg, ≈80% per-acid, ≡ 32mg and 0.185mmol) added, and stirred for 26.5h. Sodium thiosulfate (≈0.5g) was added, then the reaction mixture was extracted by the standard method. Column chromatography eluting with ethyl acetate : petrol, (30:70), returned starting material **(108)** (17mg, 0.0370mmol, 17%).

Following further chromatography (ethyl acetate : petrol, 40:60), a white solid was obtained (45mg) which contained *ent*-1α,10β-dihydroxy-3α,13-diacetoxy-2β-methyl-20-norgibberell-16-ene-7,19-dioic acid 7-methyl ester 19,10-lactone **(109)**, albeit contaminated with *m*ClBA, (which was removed on recrystallisation), ≡33mg lactone **(109)**, 0.0695mmol, 32%; R_f = 0.49 (ethyl acetate : petrol, 50:50); mp 104-106°C (from chloroform and petrol); (Found C, 62.8; H, 7.4%; M^+, 476.2046. $C_{25}H_{32}O_9$ requires C, 63.0; H, 6.9%; M, 476.2046); υ_{max}/cm^{-1} * 1734 (C=O); δ_H 1.01* (3H, d, *J* 7, 2-CH_3), 1.02* (3H, s, 18-H_3), 2.03 and 2.19 (each 3H, each s, each O_2CCH_3), 2.72 (1H, d, *J* 11, 6-H), 3.48 (1H, d, *J* 11, 5-H), 3.73* (3H, s, OCH_3), 3.74* (1H, m, 1-H), 5.00 (1H, br s, 17-H), 5.08 (1H, d, *J* 4, 3-H) and 5.17 (1H, br s, 17-H) [*signals overlap]; δ_C 14.41 (C-18), 16.63 (C-11), 20.80, 21.05 and 22.10 (2α-CH_3, 2 x $O_2C\underline{C}H_3$), 34.32 (C-2), 36.35, 40.00 and 42.26 (C-12, C-14, C-15), 46.29 and 53.27 (C-4, C-8), 47.62, 50.10, 50.57 and 52.26 (C-5, C-6, C-9, OCH_3), 68.64 (C-1), 73.94 (C-3), 84.35 (C-13), 94.64 (C-10), 108.26 (C-17), 153.66 (C-16), 169.98 and 170.10 (2 x $O_2\underline{C}CH_3$), 172.52 (C-7) and 176.52 (C-19); *m/z* 476 (M^+, 44%), 445 (14), 435 (27), 434 (100), 416 (39), 374 (49), 359 (84), 358 (80), 298 (74) and 235 (98).

Further elution (ethyl acetate : petrol, 40:60), gave the epoxide **(112)**, (4mg, 0.00813mmol, 4%), as a pale yellow gel; R_f = 0.12 (ethyl acetate : petrol, 50:50); (Found M^+, 492.1976. $C_{25}H_{32}O_{10}$ requires M, 492.1996); δ_H (300 MHz) 1.02* (3H, s, 18-H_3), 1.03* (3H, d, *J* 7, 2-CH_3), 2.01 and 2.19 (each 3H, each s, each O_2CCH_3), 2.74* (1H, d, *J* 5.5, 17-H), 2.75 (1H, d, *J* 11, 6-H), 3.13 (1H, d, *J* 5.5, 17-H), 3.48 (1H, d, *J* 11, 5-H), 3.73 (3H, s, OCH_3), 3.76 (1H, d, *J* 5, 1-H) and 5.09 (1H, d, *J* 3.5, 3-H) [*signals overlap]; δ_C 14.38 (C-18), 17.14 (C-11), 20.80, 21.62 and 21.79 (2α-CH_3, 2 x $O_2C\underline{C}H_3$), 33.24, 40.54 and 42.70 (C-12, C-14, C-15), 34.26 (C-2), 46.26 and 53.27 (C-4, C-8), 48.32, 48.67, 51.43 and 52.38 (C-5, C-6, C-9, OCH_3), 66.92 (C-17), 68.73 (C-1), 73.91 (C-3), 77.50 (C-16), 80.58 (C-13), 94.48 (C-10), 169.91 and 170.07 (2 x $O_2\underline{C}CH_3$), 172.23 (C-7) and 176.29 (C-19); *m/z* 492 (M^+, 7%), 462 (5), 461 (9), 451 (24), 450 (100), 449 (29), 432 (14), 420 (11), 393 (56) and 315 (40).

Further elution (100% ethyl acetate) gave a brown liquid, a mixture of compounds which appeared, following ^1H and ^{13}C NMR and MS analysis, to have lost the acetate protecting groups on one or both of the alcohols (40mg).

ent-13-Acetoxy-10β-hydroxy-2-methyl-1-oxo-20-norgibberella-2,16-diene-7,19-dioic Acid 7-Methyl Ester 19, 10-Lactone (120)

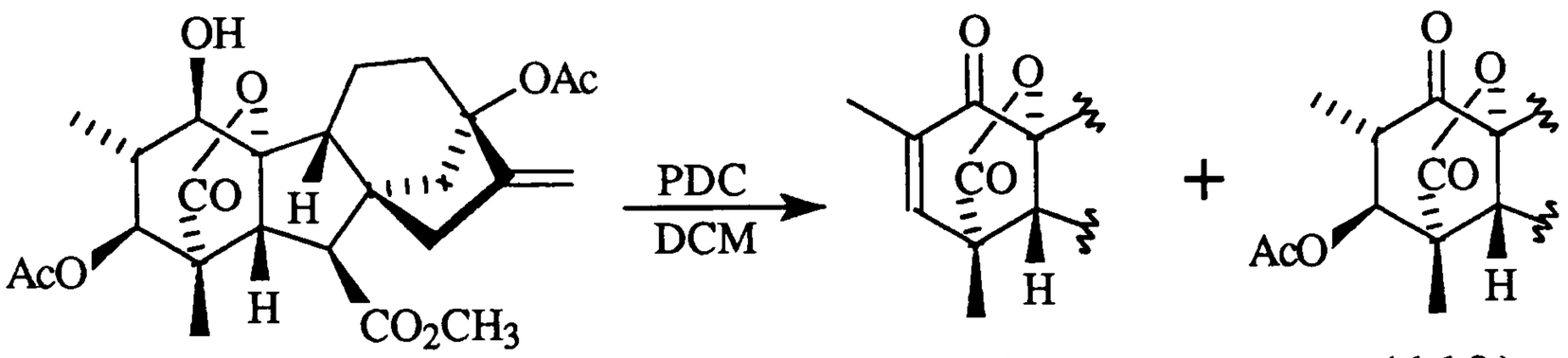


(109) **(120)**

The diacetylated alcohol **(109)** (33mg, 0.0695mmol) was dissolved in acetonitrile (10ml) at 0°C and TPAP (1.6mg, 0.00455mmol) added, followed by NMO (22mg, 0.188mmol). The mixture became green-brown, and after 2.25h was filtered through silica on Celite©. ^1H NMR spectra analysis of the crude product indicated an eliminated compound **(120)** was present. Following column chromatography (ethyl acetate : petrol, 20:80), the α,β-unsaturated ketone **(120)** was obtained as an off white solid, (24mg, 0.0580mmol, 83%). Attempted recrystallisation failed. R_f = 0.79 (ethyl

acetate : petrol, 50:50); (Found M^+, 414.1674. $C_{23}H_{26}O_7$ requires M, 414.1679); υ_{max}/cm^{-1} * 1689, 1735 and 1793 (C=O); λ_{max}/nm ($CHCl_3$) 262; δ_H (300 MHz) 1.31 (3H, s, 18-H_3), 1.83 (3H, d, *J* 1.5, 2-CH_3), 2.04 (3H, s, O_2CCH_3), 2.83 (1H, d, *J* 10.5, 6-H), 3.38 (1H, d, *J* 10.5, 5-H), 3.75 (3H, s, OCH_3), 5.01 (1H, br s, 17-H), 5.19 (1H, dd, *J* 3 and 1.5, 17-H) and 6.68 (1H, q, *J* 1.5, 3-H); δ_C (300 MHz) 14.80 (C-18), 15.25 (2-CH_3), 17.73 (C-11), 22.05 ($O_2C\underline{C}H_3$), 36.35, 40.15 and 42.34 (C-12, C-14, C-15), 47.37, 51.09 and 52.43 (C-6, C-9, OCH_3), 50.54 and 51.15 (C-4, C-8), 62.70 (C-5), 84.12 (C-13), 93.15 (C-10), 108.54 (C-17), 136.12 (C-2), 147.83 (C-3), 153.08 (C-16), 169.95 ($O_2\underline{C}CH_3$), 171.60 (C-7), 175.41 (C-19) and 190.82 (C-1); *m/z* 414 (M^+, 31%), 383 (9), 373 (23), 372 (100), 310 (71) and 251 (96).

Oxidation of the Alcohol (109) with PDC

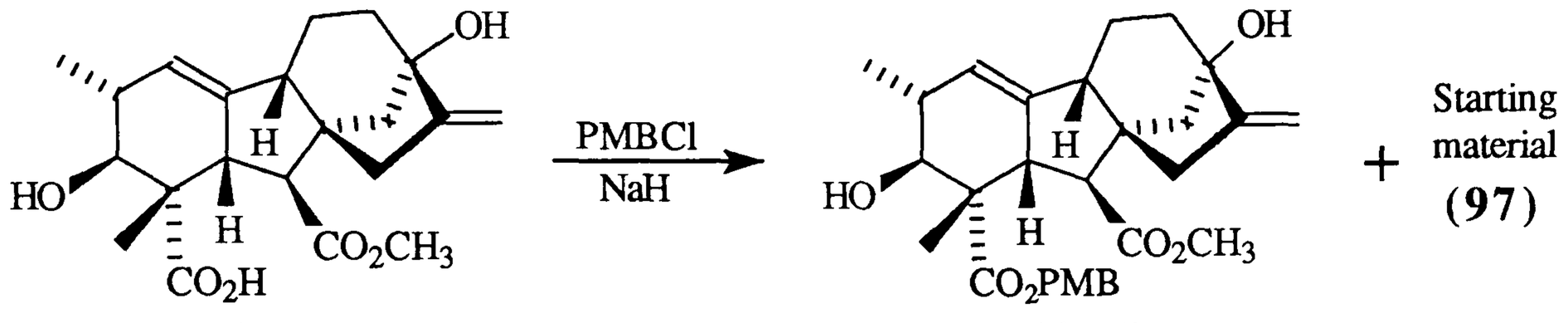


(109) **(120)** 2 : 1 **(110)**

The alcohol (**109**) (89mg, 0.188mmol) in DCM (12ml) was treated with crushed and dried 0.4nm diameter molecular sieves (≈0.7g) and PDC (96mg, 0.255mmol) at room temp. for 14h. The blackened suspension was then filtered through Florisil© on Celite© to give a yellow-brown semi solid. Following column chromatography (ethyl acetate : petrol, 20:80), a clear and colourless liquid was obtained containing an inseparable mixture by flash column chromatography of α,β-unsaturated ketone (**120**) and *ent*-3α,13-diacetoxy-10β-hydroxy-2β-methyl-1-oxo-20-norgibberell-16-ene-7,19-dioic acid 7-methyl ester 19, 10-lactone (**110**) in a ratio of 2:1 respectively (determined by ^1H NMR spectroscopy), (47mg, 0.113mmol, 60% and 26mg, 0.0555mmol, 30% respectively); R_f = 0.52 and 0.50 (ethyl acetate : petrol, 30:70). Data for 3-acetoxy ester (**110**): (Found $[MH]^+$, 475.1948. $C_{25}H_{30}O_9$ requires MH, 475.1968); υ_{max}/cm^{-1} * 1702, 1736 and 1793 (C=O); δ_H (300 MHz) 1.02 (3H, d, *J* 6.5, 2-CH_3), 1.31 (3H, s, 18-H_3), 2.03 (3H, s, O_2CCH_3), 2.15 (3H, s, O_2CCH_3), 2.56 (1H, dd, *J* 12 and 6, 9-H), 2.86

(1H, d, *J* 10, 6-H), 3.13 (1H, qd, *J* 6.5 and 5.5, 2-H), 3.46 (1H, d, *J* 10, 5-H), 3.74 (3H, s, OCH_3), 5.03 (1H, br s, 17-H), 5.18 (1H, m, 17-H) and 5.45 (1H, d, *J* 5.5, 3-H); δ_C (300 MHz) 8.80 (2α-CH_3), 13.73 (C-18), 17.29 (C-11), 20.51 and 21.94 (2 x $O_2C\underline{C}H_3$), 36.13, 40.08 and 42.17 (C-12, C-14, C-15), 43.26, 45.99, 51.27 and 54.61 (C-5, C-6, C-9, OCH_3), 50.19 and 53.37 (C-4, C-8), 60.40 (C-2), 74.60 (C-3), 84.00 (C-13), 95.00 (C-10), 108.63 (C-17), 152.95 (C-16), 169.88 and 171.52 (2 x $O_2\underline{C}CH_3$), 171.59 (C-7), 175.73 (C-19) and 202.27 (C-1); *m/z* (CI) 475 ($[MH]^+$, 13%), 474 (9), 432 (8) and 311 (100).

ent-3α,13-Dihydroxy-2β-methyl-20-norgibberella-1(10),16-diene-7,19-dioic Acid 7-Methyl 19-Paramethoxybenzyl Diester (125)



(97) **(125)**

Sodium hydride (280mg, 60% in oil; ≡168mg NaH, 7.00mmol) was washed with petrol (3 x 10ml), 18-c-6 added (catalytic quantity) and THF (5ml) added and the stirred suspension cooled to 0°C. The acid **(97)** (490mg, 1.30mmol) in THF (10ml) was added to the hydride under anhydrous conditions; the reaction was allowed to reach room temp. for 1h. The mixture was again lowered to 0°C and paramethoxybenzylchloride (0.8ml, 5.90mmol). Following no change in the reaction when followed by TLC analysis at either 0°C or room temp., the reaction was heated at reflux for 15.5h; then was cooled and underwent the standard extraction treatment. Post column chromatography, (ethyl acetate : petrol, 30:70), an impure product **(125)** was obtained, a pale orange solid; mp 73-76°C (from carbon tetrachloride and petrol); (48mg, 0.0968mmol, 7%); R_f = 0.49 (ethyl acetate : petrol, 50:50); (Found M^+, 496.2450. $C_{29}H_{36}O_7$ requires M, 496.2461); δ_H 1.12 (3H, d, *J* 7, 2-CH_3), 1.33 (3H, s, 18-H_3), 2.97 (1H, m, 5-H), 3.11 (1H, d, *J* 6, 6-H), 3.67 (3H, s, 7-OCH_3), 3.71 (3H, s, Ar-OCH_3), 3.86 (1H, d, *J* 2.5, 3-H), 4.94 (2H, s, 19-OCH_2Ar), 5.00, 5.07 and 5.11 (3H,

m, 1-H, 17-H_2), 6.88 (2H, d, *J* 8, Ar-H_2) and 7.26 (2H, d, *J* 8, Ar-H_2); δ_C 17.02 (C-18), 18.51 (C-11), 22.13 (2-CH_3), 33.29 (C-2), 37.74, 39.07 and 45.74 (C-12, C-14, C-15), 45.98, 48.90, 49.31, 50.06, 50.59 and 51.78 (C-4, C-5, C-6, C-8, C-9, 7-OCH_3), 55.24 (19-OCH_2Ar), 75.65 (C-3), 79.40 (C-13), 106.33 (C-17), 113.75 (C-1), 115.67, 129.53, 139.93 and 154.18 (aromatics), 141.55 (C-10), 154.00 (C-16), 176.84 (C-7) and 180.00 (C-19); *m/z* 496 (M^+, 6%), 478 (9), 448 (2), 360 (15), 297 (12), 253 (41), 137 (14), 122 (72), 121 (100) and 85 (71).

Further elution with ethyl acetate : petrol (50:50) gave starting material **(97)**, (170mg, 0.452mmol, 35%), a pale brown solid, R_f = 0.16 (ethyl acetate : petrol, 50:50), data as previously reported.

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